Vol. 37, No. 1 March 1950

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE
CANADA AND INDIA: MACMILLAN

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TWO SPECIES OF *APHELENCHOIDES* ASSOCIATED WITH STRAWBERRY BUD DISEASE IN BRITAIN

BY MARY T. FRANKLIN

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(With 1 Text-figure)

Two species of *Aphelenchoides* have been found in nematode-diseased strawberries from several places in Britain. They are identified as *Aphelenchoides fragariae* (Ritzema-Bos, 1891) and *A. ritzema-bosi* (Schwartz, 1911) and the differences between them are fully discussed. *A. olesistus* (Ritzema-Bos, 1893) is considered a synonym of *A. fragariae* (Ritzema-Bos, 1891).

OBSERVATIONS ON THE IDENTITY OF THE EELWORMS

The strawberry can be parasitized by at least four species of nematode with which disease symptoms are associated. *Neotylenchus abulbosus* Steiner, 1931, and *Aphelenchoides besseyi* Christie, 1942, have not been recorded on strawberries in Britain. The latter is associated with the condition known as 'summer dwarf' in the southern States of the U.S.A. (Christie, 1942). When infected plants were grown in the more northerly State of Massachusetts the eelworms died after one season apparently because of the adverse climate (Christie, 1938), which suggests that this species is unlikely to occur in Britain, or to survive here if introduced. *Neotylenchus abulbosus* was found by Steiner (1931) in strawberry buds and within the leaf stalks and laminae, sometimes in discoloured patches, in plants affected with 'yellows' or 'xanthosis' from California, and also in plants from Wisconsin and Germany apparently without the 'yellows' symptoms.

The other two nematodes known to be associated with disease in strawberries are *Anguillulina dipsaci* (Kühn, 1857) Gerv. & v. Ben., 1859, and *Aphelenchoides fragariae* (Ritzema-Bos, 1891). Hodson (1938) gave the first detailed account of disease in strawberries in Britain due to *Anguillulina dipsaci*, but Massee (1913) apparently knew it on this host and described the symptoms associated with its presence. It occurs inside the leaf tissue, petioles and flower stems, and causes thickening, distortion and puckering of the invaded parts. *Aphelenchoides fragariae* was first described by Ritzema-Bos in strawberries from Kent in 1891. Since then there have been many records from all over Britain of disease in strawberries associated with nematodes identified as *A. fragariae*.

The symptoms have been given a number of descriptive names such as 'red plant', 'cauliflower', 'spring dwarf' and 'crimp'. The disease has been described by Goodey (1933), Ogilvie & Thompson (1936), Dicker (1948) and Reid (1949).

Probably symptoms have, on occasion, been attributed to *A. fragariae* which have in fact been due to other causes such as frost damage (Klinkenberg, 1947), mechanical injury, *Anguillulina dipsaci* or *Bacterium fascians* (Tilford). The severely dwarfed, distorted and galled condition described by Ritzema-Bos in the plants in which he found *Aphelenchoides fragariae* may possibly have been due at least in part to the presence of *Bact. fascians*, since they are strikingly similar to the symptoms produced in strawberry plants which Lacey (1942) inoculated with this organism.

Aphelenchoides fragariae is usually found living ectoparasitically within the tightly folded crown and runner buds, but small bunches of the eelworms may also be found at certain times on the upper surface of partly expanded leaves around the midrib, at its base. In the neighbourhood of these bunches the leaf often shows superficial damage apparently caused by the feeding of the eelworms. Older, fully expanded leaves may show small dry brown 'feeding' areas near the midrib when no eelworms are to be found, suggesting that feeding took place within the folded bud. Occasionally (Goodey, 1928; Ogilvie & Thompson, 1936) *A. fragariae* has been found living endoparasitically in the leaf tissues, generally near or within brown patches, but this is not common.

Since 1947, strawberry plants from widely separated districts in England, Scotland and Ireland showing the symptoms generally associated with eelworm attack have been examined for nematodes either by dissection of the buds in water or by soaking teased portions of the plants in water in a Baermann funnel.

Some plants received from Ayrshire in February 1947 had a fairly high population of *Aphelenchoides*. When some of the eelworms were killed by heat Dr T. Goodey observed that the males were of two distinct forms, one having the gently curved, arc-shaped tail typical of *A. olesistus*, the other the sharply hooked tail which was regarded as typical of *A. fragariae* and *A. ritzema-bosi*. Subsequently, other plants were received with *Aphelenchoides* sometimes of one type, sometimes of the other. They can be distinguished by three morphological characters. In the male the curvature of the tail when the eelworm is killed by gentle heat is quite distinct and constant. It is hooked in the *fragariae-ritzema-bosi* type and curved in the *olesistus* type. In the *ritzema-bosi* type the greatest body width is about 20-21 μ and the ratio of length to width about 40, while in *olesistus* the width is 14–15 μ and the ratio of length to width is about 50. Thirdly, eelworms of the *fragariae-ritzema-bosi* type have the excretory pore situated well behind the nerve ring, while in the *olesistus* type it is more or less at the level of the nerve ring. As the position of the nerve ring is not always easy to determine, measurements were made on a number of individuals of the two types of the distance of the excretory pore from the anterior end and the distance of the valve apparatus in the oesophageal bulb from the anterior end. The ratio of the first to the second of these distances was, on the average, 1·76 in the *fragariae-ritzema-bosi* type, and 1·38 in the *olesistus* type.

Goodey (1928) found no morphological differences between what has been regarded as *A. fragariae* in strawberries in this country and *A. ritzema-bosi* in chrysanthemums.

Nor have morphological differences been found between the more slender *olesistus*-like eelworms found in strawberries and *A. olesistus* from violet or from fern.

Specimens of the *ritzema-bosi*-like eelworms from strawberry were sent to Dr M. W. Allen of California, who in return forwarded specimens regarded as *A. fragariae* from strawberries grown in California. The latter proved to be *olesistus*-like, slender, with gently curving male tail and excretory pore close to the nerve ring. Dr Allen expressed the opinion that *A. fragariae* and *A. olesistus* were indistinguishable from one another morphologically and was inclined to think that *A. olesistus* was a synonym of *A. fragariae*. Further, he considered the English *A. fragariae* to be morphologically indistinguishable from *A. ritzema-bosi* while it could easily be distinguished from his *A. fragariae*. It thus seemed evident that two apparently distinct species of *Aphelenchoides* were being called *A. fragariae*.

Ritzema-Bos's two papers in which he described and named *A. fragariae* (1891), and *A. olesistus* (1893) were again consulted and the descriptions and drawings of the two species compared with one another and with the two types of *Aphelenchoides* found in strawberries. As a result the following conclusions, which agree with the opinions expressed by Dr Allen, are drawn:

(1) There appears to be no important morphological difference between *A. fragariae* and *A. olesistus* as described by Ritzema-Bos. He says that the mouth stylet is knobbed in *A. fragariae*, while in *A. olesistus* it is slender and thickened posteriorly but without knobs. In his drawings, however, the stylet in *A. olesistus* has a distinctly knobbed extremity, and later workers (e.g. Schwartz, 1911; Goodey, 1928) have always described it as knobbed. Three males and five females of *A. fragariae*, and six males and seven females of *A. olesistus*, were measured by Ritzema-Bos, and these measurements agree fairly well. In both species the worms are slender, most being 14–15μ broad and about forty-five to fifty times as long as broad. An important difference in Ritzema-Bos's view between the two species is the difference in their action on the host. While *A. fragariae* causes 'cauliflower' symptoms in the strawberry plant in which it lives usually as an ectoparasite, *A. olesistus* causes the destruction of the leaf tissues of begonia and *Asplenium* spp. which it invades and lives in endoparasitically. It was because of this tissue-destroying effect that Ritzema-Bos gave it the trivial name *olesistus*, from the Greek words ὀλλύναι (root ὀλεο) and ἱστος meaning 'destroy' and 'tissue'. However, nematodes, apparently of Ritzema-Bos's species *Aphelenchoides fragariae*, have been successfully transferred from strawberry to fern, in which they caused brown lesions, and Steiner (1932) claims to have transferred *A. olesistus* from ferns (presumably showing leaf-blotch symptoms) to strawberry where dwarfing was produced. The same population of nematodes may therefore produce different symptoms and adopt different modes of life in different host plants, and this character alone cannot be regarded as specific. The conclusion thus seems justified that *A. olesistus* (Ritzema-Bos, 1893) is a synonym of *A. fragariae* (Ritzema-Bos, 1891). Marcinowski (1908) also considered these two species to be identical.

(2) As the true *A. fragariae* of Ritzema-Bos is therefore a slender worm with the excretory pore near the nerve ring and the tail of the male curving gently when killed by heat, the stouter species, frequently found in strawberries in Britain, having the excretory pore farther back and the tail of the male sharply hooked cannot also be *A. fragariae.*

In his description of *A. ritzema-bosi*, Schwartz (1911) compares this species from chrysanthemum with *A. fragariae* (*olesistus*) from violet and fern and gives as the main differences the greater size of *ritzema-bosi* with its stouter mouth stylet and the very much more sharply curved male tail. He does not find that *ritzema-bosi* is relatively broader than *A. fragariae*, but the greatest breadth of the former is given as 23–24μ, and of the latter 14–16μ in eelworms from violets and 12–14μ in eelworms from ferns. He does not mention the position of the excretory pore in relation to the nerve ring, but gives the distance of the excretory pore from the anterior end of the worms and values for the ratio of total length of the worms divided by the distance of the excretory pore from the anterior end. He could apparently observe the excretory pore in only nine of twenty-one adult females and in none of the ten male worms from chrysanthemum. In the nine females the ratio of total length to distance of excretory pore from the anterior end averaged 9, while the same ratio in eelworms from violets was 6 in twenty-one females and 5 in fourteen males: in worms from fern it was 8 and 6 in twenty females and ten males respectively. Apparently, therefore, in Schwartz's species, *A. ritzema-bosi*, the excretory pore was more difficult to find than in the other species he examined, and he does not give sufficient figures for a valid comparison to be made of its position with that in the other species. Thus, the greater size and the sharply hooked male tail serve to distinguish *A. ritzema-bosi* from true *A. fragariae*, and these same two points distinguish the stouter form of *Aphelenchoides* found in strawberry in Britain from the true *A. fragariae*; the former must therefore be regarded as *A. ritzema-bosi.*

Ritzema-Bos (1891) described a second species of *Aphelenchoides* from strawberry and named it *A. ormerodis.* He gives measurements of only two males and one female and states that the eelworms were about the same length as his *A. fragariae*, but were twice as wide, the ratio of length to breadth being 23, 26 and 27 respectively. The mouth stylet is said to be rather larger than in *A. fragariae*, but has no clear knob at the posterior end. The excretory pore is described as being close to the beginning of the intestine, and in the drawings it appears to be almost as far forward as it is in *A. fragariae.* The description is rather unsatisfactory in some points, but it seems fairly certain, from the absence of knobs on the stylet and the position of the excretory pore, that the species described is not the same as the one later described by Schwartz as *A. ritzema-bosi.* Schwartz himself studied Ritzema-Bos's description and came to the conclusion that his chrysanthemum nematode was not Ritzema-Bos's *A. ormerodis.*

Steiner (1932) claims to have transferred *A. ritzema-bosi* from chrysanthemums

to strawberries where dwarfing symptoms were produced. The fact that nematodes both from chrysanthemums and from ferns (in which the parasite has always been recognized as *A. olesistus*, now *fragariae*) were transferred by Steiner to strawberries and produced symptoms in them does not prove that the nematodes were necessarily of the same species, but it supports the conclusion that the strawberry may harbour both *A. fragariae* and *A. ritzema-bosi*. Junges (1938) also found these two species living together in the same host, *Aster alpinus* var. *altaicus*, just as they do in strawberry, except that in *Aster* they were both endoparasitic, causing blotching of the leaves.

Both species cause leaf-blotch symptoms in a number of hosts—*Aphelenchoides fragariae* in ferns and lilies, *A. ritzema-bosi* in chrysanthemums, dahlias, etc.—but in strawberry they are normally ectoparasitic, giving rise to various malformations and often causing the death of buds. Junges (1938) observed the symptoms caused by *A. fragariae* (*olesistus*) in some forty different plants and, in all cases but one, brown blotches were produced on the leaves. The one exception was *Potentilla alpina* hybr., apparently the only representative he observed of the family Rosaceae, to which the strawberry belongs, and a genus which is closely related to *Fragaria*. In this plant the symptoms showed a striking similarity to the cauliflower disease of strawberry as described by Ritzema-Bos, but in addition to the dwarfing and fasciation there were small blotches on the leaves.

Observations on *Aphelenchoides* spp. found naturally infesting strawberry plants in Britain

The Ayrshire plants received in February 1947, in which the two species of *Aphelenchoides* were first noticed, were planted in pots, and fresh eelworms were extracted from them in April 1948. All the males observed this time had the gently curved type of tail and were apparently *A. fragariae*. Eleven males varied in length from 0·693 to 0·864 mm. (mean 0·764 mm.), and twelve females varied from 0·634 to 0·862 mm. (mean 0·765 mm.). In breadth the male worms were 13–15 μ (mean 14 μ) and the females 11–16 μ (mean 14 μ), giving mean values for the ratio of length to breadth of 56 for the males and 55 for the females.

Another sample of strawberry plants received from Perthshire early in 1947 contained a population typical of *A. ritzema-bosi*. The average length of eleven males was 0·865 mm. (varying from 0·747 to 1·01 mm.) and of twelve females 0·838 mm. (varying from 0·729 to 0·972 mm.). The average breadth was 21 μ in both sexes (18–23 μ in males, 18–28 μ in females), and the mean ratio of length to breadth was 42 in the males and 40 in the females. The tails of the males became sharply hooked on killing by heat. The position of the excretory pore was found to be in the region of the nerve ring in the former (*A. fragariae*) population and well behind the nerve ring in the latter (*A. ritzema-bosi*). These two species of *Aphelenchoides* may therefore occur in the strawberry, occasionally at the same time.

By courtesy of Mr R. D. Reid of Auchincruive, Scotland, several other samples of

strawberries showing signs of eelworm disease were received. Some plants from Carluke, Lanark, received in October 1947, contained both *A. fragariae* and *A. ritzema-bosi*, while *A. ritzema-bosi* alone was found in plants from Stonehouse, Lanark (December 1947), and from Perthshire (March 1948). A plant sent from Starcross, Devon, in April 1948, showing typical 'red plant' symptoms contained *A. fragariae*. In May 1948 the strawberry-growing district of the Tamar valley on the borders of Devon and Cornwall was visited, and plants showing various symptoms and degrees of eelworm disease were collected for examination. In four samples *A. fragariae* was found, while in four others *A. ritzema-bosi* was the parasite. On one farm plants from one field contained one species, while plants from another field contained the other. At about the same time *A. ritzema-bosi* was found in strawberry plants grown in Hertfordshire. In September 1948 *A. fragariae* was found in plants from Cambridgeshire, and in the following month *A. ritzema-bosi* was found in some plants from eastern Scotland. In the spring of 1949 some plants received from Dublin, showing symptoms of the 'red plant' type, were found to contain *A. fragariae*.

In all these cases the eelworms were found within the crown or runner buds, living ectoparasitically amongst the hairs and between the folded leaves, but on two occasions in 1948 isolated leaves were received (from Kent in June and from an Auchincruive greenhouse in November) which were partly expanded and both had on the upper surface a small cluster of eelworms at the base of the midrib. The eelworms were quiescent but not desiccated, forming a fairly loose cluster, apparently adhering together because of a slight stickiness. The leaves showed slight superficial brownish lesions in 'feeding areas' close to the worms. In both cases the eelworms were of the type now recognized as *A. ritzema-bosi*.

A few isolated strawberry leaves received from Cornwall in 1947 had small blackish brown patches in or close to which were found nematodes, which were apparently of the *A. ritzema-bosi* type living endoparasitically, but this was the only instance during these investigations in which the worms were found living endoparasitically.

It therefore seems that both *A. fragariae* and *A. ritzema-bosi* are widespread in strawberries in Britain. Both this fact, and the somewhat inadequate original descriptions of the species, have probably played a part in the confusion over their identity.

The main points of difference are set out in Table 1, and are represented diagrammatically in Fig. 1.

INOCULATION EXPERIMENTS

The morphological similarity between *A. fragariae* and the fern leaf-blotch nematode has led workers to attempt inoculation experiments to prove whether these are really one or two species. Marcinowski (1908) succeeded in setting up disease symptoms consisting of brown blotches on the leaves of strawberries which she had inoculated with pieces of diseased *Cypripedium* leaves. The result of this experiment and the similarity in morphology between the nematodes which she found in

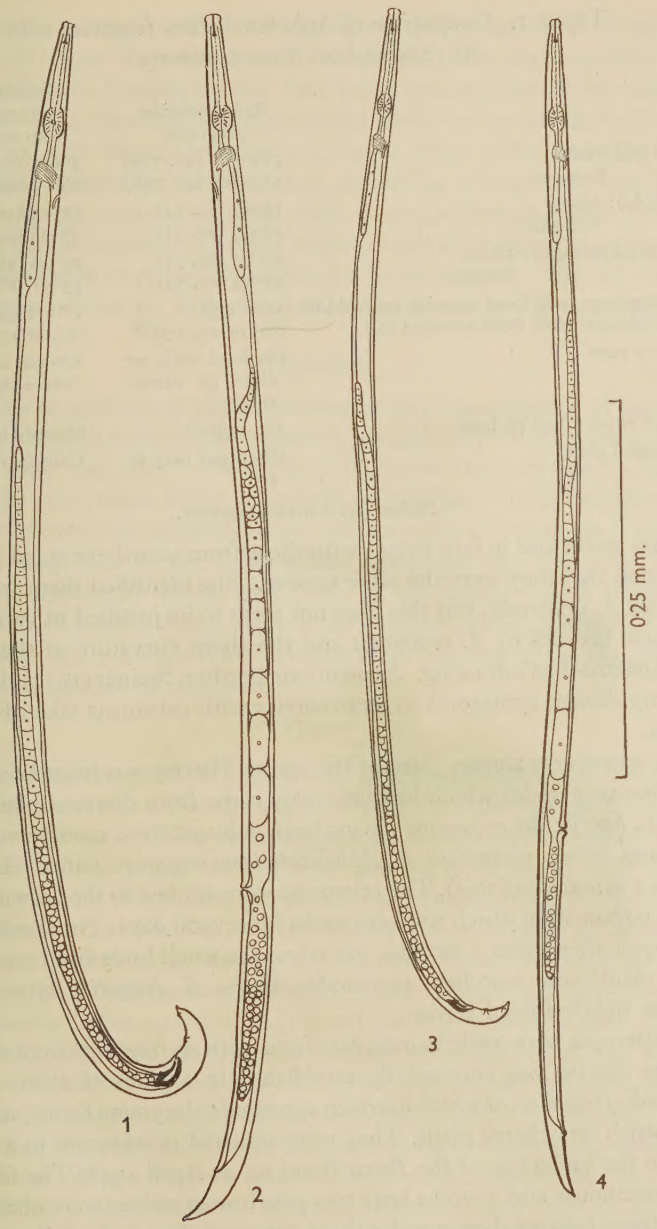

Fig. 1. The two species of *Aphelenchoides* from strawberry showing the difference in width, in the position of the excretory pore and in the curvature of the male tail. 1, *A. ritzema-bosi*, male; 2, *A. ritzema-bosi*, female; 3, *A. fragariae*, male; 4, *A. fragariae*, female.

TABLE 1. *Comparison of* Aphelenchoides fragariae *with* A. ritzema-bosi *from strawberry*

	Aphelenchoides fragariae	*Aphelenchoides ritzema-bosi* from strawberry
Length (μ): Males	573–864 (av. 732)	566–1010 (av. 787)
Females	552–886 (av. 796)	668–1020 (av. 817)
Breadth (μ): Males	12–17 (av. 14)	15–23 (av. 20)
Females	12–17 (av. 15)	17–28 (av. 21)
Ratio length/breadth: Males	40–63 (av. 51)	29–46 (av. 40)
Females	36–63 (av. 53)	35–51 (av. 40)
Ratio excretory pore from anterior end/middle of oesophageal bulb from anterior end	1·22–1·33 (av. of 19, 1·38)*	1·61–1·94 (av. of 17, 1·76)*
Excretory pore	On level with, or close to, nerve ring	Always well behind nerve ring
Male tail when killed by heat	Arc-shaped	Sharply hooked
Oesophageal gland	Often not easy to see	Usually fairly obvious

* Males and females together.

Cypripedium leaves and in fern fronds with those from strawberries led Marcinowski to the opinion that they were the same species. She identified them with Ritzema-Bos's species *A. ormerodis*, but this does not seem to be justified in view of the very much greater breadth of *A. ormerodis* and the sharp curvature of the male tail as shown in Ritzema-Bos's drawing. As mentioned earlier, Steiner (1932) also succeeded in setting up disease symptoms in strawberries with eelworms taken from infested fern fronds.

A young strawberry runner plant of the variety Huxley was inoculated in January with 100–200 nematodes which had originally come from diseased plants of *Lilium regale* and *L. henryi*, the eelworms having been cultured for 4 months on an artificial medium, and being identified as *Aphelenchoides fragariae* (since *A. olesistus* is regarded as a synonym of this). The eelworms were applied to the crown of the plant in a wad of cotton-wool which was kept moist for several days. No disease symptoms had developed after about 5 months, but when the small buds from a young runner from this plant were searched, nematodes of the *A. fragariae* type were found amongst the tightly folded leaves.

Several attempts were made to inoculate ferns with *A. fragariae* from strawberries. In one case disease was successfully established in a plant of *Pteris* sp. Twenty *Aphelenchoides fragariae*, of which fourteen appeared to be young forms, were obtained from a Scottish strawberry plant. They were squirted in water on to a small damp pad fixed to the underside of the *Pteris* frond on 21 April 1948. The fern was kept in a cool greenhouse and 5 weeks later two pale brown stripes were observed on the frond and these became darker and a third appeared during the following 3 weeks. The stripes broadened and had begun to coalesce when the plant died during a sudden hot spell about 3 months after the inoculation. The affected part of the dead

frond was teased in water and it was estimated that about sixty to seventy dead eelworms in all stages of development were present, thus showing that the nematodes had established themselves and were reproducing. One of two other inoculations carried out in the same way at about the same time on a different frond of the same plant, but with nematodes from a diseased violet plant, was also successful. A brown stripe appeared on the frond and, on teasing it about 3 months later, after the death of the plant, twice or three times as many eelworms were found as had been used for the inoculation. No other lesions of this type appeared on the plant and the inoculated fronds were well separated from one another. Several unsuccessful attempts to infect *Pteris* sp. with *Aphelenchoides ritzema-bosi* from strawberry were made.

Symptoms

The symptoms in strawberry plants associated with the presence of nematodes of the genus *Aphelenchoides* are of two main types and may be referred to broadly as 'cauliflower' and 'red plant'. Now that it is known that two species of *Aphelenchoides* may be concerned, either separately or together, it may possibly be found that the type of symptom depends upon which species of nematode is present.

In the two cases seen by the writer where nematodes were found on the surface of half-grown strawberry leaves they were of the species which would now be identified as *A. ritzema-bosi*, but there is no evidence to suggest that *A. fragariae* never occurs in this position. The 'feeding areas' on the leaves can probably be caused by either species of nematode.

Hosts

The recognized host range of *A. fragariae* is quite considerable now that *A. olesistus* is regarded as a synonym, but the possibility of the existence of biological races must be borne in mind. The same applies to *A. ritzema-bosi*: many hosts have been recorded for this nematode but there is little evidence to show whether or not strawberry can be attacked by populations of eelworms from all the other hosts.

When a blackcurrant seedling was inoculated with a number of *A. ritzema-bosi* from strawberry, brown lesions appeared at the bases of the petioles of the expanding terminal bud where the eelworms had been placed. The central unexpanded leaflets of the bud became brown and dropped off and the bud eventually died. The remaining parts of the plant were not searched because it was desired to find out whether the worms could establish themselves on it. The terminal bud appeared to have been killed by the nematodes, recalling the kind of damage done by *A. ribes*. In another experiment nematodes identified as *A. ribes*, from blackcurrant bushes, were inoculated on to a strawberry plant in the same manner as before, and were found after 3–4 months to have established themselves in the buds, although there were no symptoms of damage except for an irregularity in the margin of one of the leaves which may or may not have been due to the presence of the nematodes. These two inoculations, taken with the morphological similarity between *A. ritzema-bosi* and *A. ribes*,

suggest that the two species are identical and that *A. ribes* should be regarded as a synonym of *A. ritzema-bosi*, and blackcurrant as a host of the strawberry eelworm, a suggestion which has also been put forward by Dr M. W. Allen (*in litt.*). The evidence, however, is hardly sufficient at present to justify reaching a definite conclusion on this point.

The writer is grateful for collaboration and material from a number of people, in addition to those mentioned in the text. The exchange of specimens with Dr M. W. Allen provided the clue to the revised identification of the nematodes, and grateful acknowledgement is made to this nematologist for freely giving his views and allowing them to be used and published.

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(*Received* 27 *April* 1949)

EXPERIMENTS ON THE CONTROL OF CHRYSANTHEMUM EELWORM (*APHELENCHOIDES RITZEMA-BOSI*, SCHWARTZ) BY HOT WATER TREATMENT

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(With Plates 1 and 2 and 1 Text-figure)

The minimum times required for killing the chrysanthemum eelworm (*Aphelenchoides ritzema-bosi*, Schwartz) in water, over a range of temperatures have been determined and the technique employed is described. A temperature of 115° F. for 5 min. is preferable to the usual standard treatment of 110° F. for 20–30 min. and enables stools to be treated with less retardation of cuttings or death of stools, with a consequent great increase in the number of cuttings obtained. The results are of special value in connexion with varieties susceptible to damage by the hot water treatment.

Introduction

Smith (1926) states that Marcinowski's recommendation (1909) for the control of chrysanthemum eelworm (*Aphelenchoides ritzema-bosi* Schwartz) was immersion in hot water at 112° F. for 5 min. This was found to be too high a temperature for chrysanthemum cuttings even when the period of immersion was as short as 2 min.

Edwards (1934) states that a period of 10 min. at a temperature of 110° F. proved lethal to the eelworms; the technique for obtaining this information is not described. Experimenting with infested stools he found that treatment of 110° F. for 10 min. failed to give control; when the time was increased to 20 min. control was still only in the region of 60%; 30 min. treatments were successful. A retardation of some 7 days in the production of cuttings occurred with the 10 min. treatments; retardation was increased to 14 days with 20 min. treatments. Edwards recommended an immersion period of 15 min. at a temperature of 110° F., in order to overcome the retardation of the cuttings, the treatments to be carried out when the cuttings were ready to be taken.

Hodson (1933) recommended that treatments should be carried out for 20 min. if the stools were small, or 30 min. for moderate or large stools. Other workers have followed these recommendations. Goodey (1933) found that the aerial portions of rooted cuttings could be treated at a temperature of 112° F. for 5 min., keeping the roots from drying, and that root stocks would stand treatment for 5–10 min. at 122° F.

Christie & Crossman (1935) determined the water temperatures lethal to 'the begonia, chrysanthemum and strawberry strains of the nematode, *Aphelenchoides fragariae*'. They quote Brooks (1931) as recommending a period of 20 min. at a

temperature of 118·4° F. for the treatment of strawberry runners. Christie & Crossman found that a period of 55 min. at 110° F. killed all nematodes, though some worms survived after treatments of 30 and 45 min. By means of a 35 × 10 mm. test-tube, floated by a cork in the water of a constant temperature bath, they were able to treat some fifty eelworms at a time. After treatment the worms were placed in shallow watch-glasses and kept at room temperature for 6 days; some survivals were noted up to 4 days after treatment. They found that the chrysanthemum eelworm was killed in 12 min. at 115° F., in 3 hr. at 110° F. and taking a longer time with decreasing temperatures down to 100° F. when 30 hr. were required. The time required for killing all the eelworms varied with the different strains. At a temperature of 110° F. the begonia strain required 25 min., the strawberry no. 1 strain 8 hr., the strawberry no. 2 strain 40 min., and the chrysanthemum strain 3 hr. In view of previous work by the author (Staniland & Barber, 1937) and because of lack of exact information of the methods employed by Edwards (1934) for the determination of the lethal period of chrysanthemum eelworm in hot water, further work on this aspect of the problem was undertaken.

TECHNIQUE

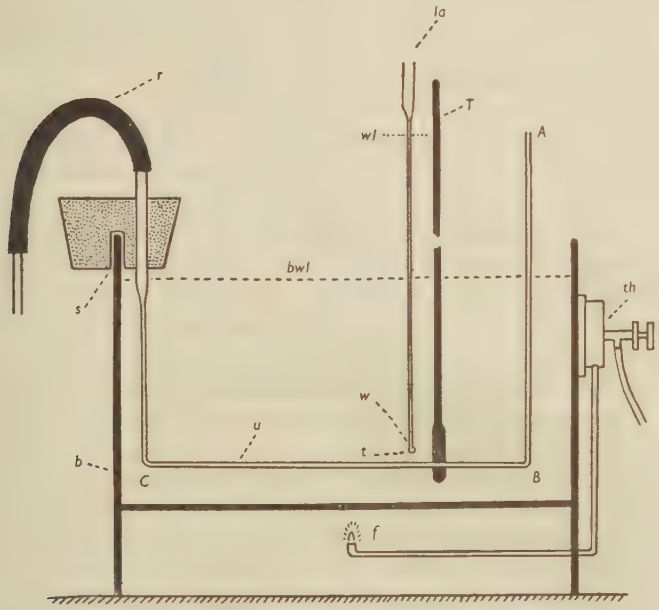
The technique employed has also been used in the determination of lethal periods for the stem and bulb eelworm (*Anguillulina dipsaci*, Kühn). The bulk of the water in which the eelworms are contained during the treatment should be as small as possible, so as to obtain a complete equilibrium between the temperature of the water and eelworms and the surrounding water in the bath at the earliest possible moment. The time taken for penetration of heat to any portion of a chrysanthemum stool is short. Successful treatment turns mainly on the time required to kill chrysanthemum eelworm at 110° F. and the safety and success of the treatment lies in applying this information to the treatment of stools regardless of size; an accurate thermometer and adequate circulation of water are presumed.

The treatment of eelworms in vitro *in water of different temperatures*

The apparatus used (Text-fig. 1) was a copper water-bath, *b*, heated by a gas flame, *f*: the temperature of the bath being kept constant by means of a thermostat, *th*. The eelworms were treated in water in a glass capillary U-tube, *u*. The undrawn portion of this tube passed through a large cork, with a slit, *s*, cut in it; by this means the cork could be clipped on to the side of the bath. To the glass tube was attached a length of rubber tubing, *r*, with a glass mouthpiece. The amount of water in the bath was so arranged that the bulk of the capillary portion of the tube was well below bath-water level, *bwl*. A thermometer, *T*, was placed so that the bulb lay close to the bottom arm of the capillary tube.

Supplies of eelworms were obtained from dried infested leaves by means of a Baermann funnel from which they were run into a watch-glass. The length and internal bore of the arm *AB* of the capillary U-tube was so arranged by trial that,

when filled with water, it contained just enough to fill the length *BC*, the final position of which was horizontal. Thirty eelworms at a time were treated. This number was removed from the stock in the watch-glass with a capillary pipette, and each individual was observed to be alive and active. The worms were then collected together into a small volume of water, a little more than the capacity of the arm *AB* of the capillary U-tube. The top of the U-tube, at *A*, was then dipped into the water near the eelworms, which were all drawn into the arm *AB*. As soon as the level of the water reached the bend at *B*, the tube was removed from the water. The operation was carried out under a binocular microscope to ensure that all the worms



Text-fig. 1. Diagram of apparatus used for the determination of lethal periods, at various temperatures, for the killing of eelworms in water.

entered the tube. The U-tube was then turned so that the arm *BC* was horizontal and the water containing the worms now filled that portion.

The temperature of the bath being steady, the tube was immersed in the water for the prescribed time and the water was stirred frequently with a small wooden paddle. After treatment the water and worms were ejected into a watch-glass containing a small quantity of water, by blowing through the tube, *r*. The capillary tube was then washed through by pouring water into the glass mouthpiece and blowing again.

At the higher temperatures, where the length of treatment was only of the order

of 2 min. or less, a slightly different treatment was employed. A piece of small-bore glass tube was drawn out into a capillary tube, as shown at *la* in Text-fig. 1. The eelworms were placed in water in a watch-glass under a binocular microscope, as before, and the capillary was then used to pick up the requisite number of worms, being then moved into an area of the water free of worms; in this way water, without worms, filled about the bottom inch of the tube. The tip, *t*, of the capillary was then sealed in a bunsen flame, the water, *w*, preventing heat from reaching the worms in the few seconds necessary for this operation. The capillary was then set aside for a short while in a vertical position, so that the worms sank to the bottom. The tube was then placed in the water-bath so that the bottom was close to the thermometer bulb, the water being stirred as before. The water level, *w*, of the capillary is indicated. After the treatment the tip of the capillary was snapped off and the worms blown into water in a watch-glass for subsequent observation.

At even higher temperatures, where the purpose was to discover the temperature at which the eelworms were killed 'instantaneously', the time allowed was 10 sec., this being sufficiently short for all practical purposes; the lowest temperature at which death took place in 10 sec. was found and this was called the 'thermal death point'. The water int his case was heated in a 1500 c.c. beaker, the temperature being allowed to rise several degrees higher than the temperature to be tested. With constant stirring the water was then allowed to cool until the required temperature was reached when the capillary tube was placed in water as before. This method reduced any possible error from ascending currents of hotter water; the large bulk of water gave a slow rate of cooling and no temperature change could be observed in 10 sec.

The watch-glasses containing the treated worms were covered to prevent evaporation and kept at room temperature for 4 days, and observations were made each day to see whether any displayed activity. On the fifth day the watch-glasses were placed in a rack over the hot-water bath for periods of up to 2 hr. for, even at this stage, gentle warmth sometimes produced signs of activity; in cases of doubt this process was again carried out on the sixth day. Throughout the whole of the 6 days observations were also made on control (untreated) worms placed under similar conditions. It was found that individual eelworms displayed greater resistance to the heat treatment than the majority. The times taken to kill at the various temperatures, in all cases, apply to the most resistant worms in the samples treated. Further, the times for killing eelworms at the various temperatures were determined to the nearest minute and always to the higher figure rather than the lower. On plotting the results a reasonably smooth curve is obtained. A 'bracketing' method was used to fix the time for any particular temperature, e.g. 20 min. was found to be too short a time at 110° F. As a result, a period of 40 min. was tried; all worms were killed. Next 30 min. was tried and all worms were killed; then 25 min., when they all lived. Periods of 28 and 29 min. resulted in some worms surviving; 30 min. was again tested and found to be the minimum time at a temperature of 110° F. The treatment of worms was always carried out the day after material was placed in the Baermann

funnel and no worms were treated unless they displayed definite activity before treatment. Material from the same source was used throughout. The thermometer used was carefully checked against an N.P.L. tested thermometer for each of the temperatures on which information was sought.

Results

The results are shown in Table 1. The temperatures for which time values were obtained were from 109° to 110·5° F. in steps of 0·5° F., values were also obtained for 111°, 112°, 115° and 120° F. The temperature of 115° F. was selected as being likely to require a shorter period of treatment than at 110° F. and yet not so short as to be beyond reasonable control by growers.

Table 1. *The killing of chrysanthemum eelworm in water*

Temperature (° F.)	Time
126	Thermal death point (10 sec.)
120	1 min.
115	4 min.
112	11 min.
111	15 min.
110·5	20 min.
110	30 min.
109·5	46 min.
109	76 min.

Experiments on the application of the results to the treatment of stools

1947 *experiments*

During the season some preliminary batches of stools were treated at varying temperatures and times, at 110° F. for 30 min., 112° F. for 12 min., 115° F. for 5 min. and 130° F. for 1 min. The stools were thoroughly washed before treatment and plunged into cold water immediately after removal from the bath.

Better results were, in general, obtained at the higher temperatures and the shorter times, but complications arose because some varieties are more susceptible than others to hot-water treatment. This factor was therefore taken into account in the 1948 experiments.

1948 *experiments*

A selection was made of varieties which had proved difficult to treat at the usual temperature of 110° F., viz. Enton Beauty, Incurved Sanctity, Balcombe Supreme, Loveliness, Lilac Loveliness and Empire White. Varieties which were found to be very amenable to hot-water treatment were also included; these were Rose Adonis, Golden Coralie and Gladys Paine.

All were treated at 110° F. for 30 min., 115° F. for 5 min. and 130° F. for 1 min. The bath used was heated by a steam coil and the thermometer was checked for

accuracy. After treatment was completed the stools were planted in boxes; each 22 × 12 in. containing fifteen stools. Treatments were made on 13 and 29 November.

Results

Within a week of the treatment it was obvious that stools treated at the higher temperature for the shorter periods were better than those which had received the standard treatment. Cuttings beginning to grow were those treated at 115° F. for 5 min. while the standard treatments remained dormant. This was very noticeable with the difficult varieties treated at the earlier date.

A final assessment of the experiment was made on 10 February, by which time the differences were striking and showed well in photographs (Pls. 1 and 2). The effects on the different varieties were as follows:

Enton Beauty

Treated 13 November (Pl. 1, fig. 1). 110° F./30 min. Nearly two-thirds of the stools were killed and very few cuttings were being produced on the survivors. 115° F./5 min. All stools alive and producing numerous cuttings of great vigour. 130° F./1 min. Slightly better than those treated at 115° F.

Treated 29 November (Pl. 1, fig. 2). A very marked difference as compared with the treatments on 13 November. Those treated at 115° and 130° F. were definitely better than the standard treatment, less stools being killed, but none of the treatments produced any worthwhile cuttings.

Incurved Sanctity, Loveliness and Lilac Loveliness

The results of the three types of treatment, at the two dates, were as described for Enton Beauty. Serious loss of Loveliness stools had taken place in the previous year with the standard treatment. With Sanctity the treatment at 115° F. was better than at 130° F. (Pl. 2, figs. 1–3).

Empire White

This has always proved to be an exceptionally difficult variety. Treatment at 115° F. gave the best results, but they were not as satisfactory as with the previous varieties. The early treatments were again better than those carried out at the second date.

Balcombe Supreme

Known from past experience to be a difficult variety to treat. The only treatment carried out in 1948 was 115° F. for 5 min. Exceptionally good results were obtained. Similar results were obtained with the variety Solidity.

Rose Adonis, Golden Coralie and Gladys Paine

These are easy varieties to treat and there was no noticeable difference between the results obtained at any of the three temperatures; the earliest treatment was still preferable.

Discussion

The usually recommended temperature of 110° F. appears to have been selected as suitable for chrysanthemums largely because it has given satisfactory results with narcissus bulbs and accurate information as to lethal temperatures and times have not been available. While the use of hot-water treatment has undoubtedly been of great value to chrysanthemum growers, growers have tended to dispense with the treatment when their stocks had reached a reasonable state of health, because of the severe retardation of cuttings and serious damage which have occurred in certain varieties.

It is recommended that a temperature of 115° F. for 5 min. should take the place of the old standard treatment of 110° F. for 20–30 min. If, however, 110° F. is used with varieties which respond satisfactorily to the treatment it should not be for less than 30 min.; a treatment period of only 20 min. is not long enough to kill all the eelworms. Treatments should be as early as possible, having regard to variety and season.

Treatment for 5 min. at 115° F. has advantages with all varieties, whether easily injured by hot-water treatment or not, for it saves time, more stools can be treated at the earlier and more satisfactory dates and it is easier to maintain a bath at a steady temperature for 5 min. than for half an hour. Treatment of stools at temperatures higher than 115° F., despite the good results obtained, is not regarded as being a good commercial practice to adopt as accuracy is not easy.

The figure for the thermal death point, 126° F., is of value, since water maintained at any temperature in excess of this figure may be used for rapid sterilization of pots, boxes, etc., by plunging.

The stools should be cut down to a length of some 8–9 in., or longer according to variety and this practice is becoming widely accepted as giving the best results. Fox Wilson (1946) has dealt fully with this and other aspects of the hot-water treatment of chrysanthemums.

Special thanks are due to F. Rowe, Esq., Rylands Nurseries, Wellington, Somerset, for the great interest and help given in connexion with these experiments, for placing all the facilities of his nursery at our disposal and for giving much of his personal time. The help of assistants in the author's department is also acknowledged.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Chrysanthemum variety—Enton Beauty, treated 13 November; left to right, 110° F. for 30 min., 115° F. for 5 min., 130° F. for 1 min.

Fig. 2. Enton Beauty, treated 29 November; left to right, 110° F. for 30 min., 115° F. for 5 min., 130° F. for 1 min.

PLATE 2

Fig. 1. Chrysanthemum variety—Sanctity, treated 13 November; 110° F. for 30 min.

Fig. 2. Sanctity, treated 13 November; 115° F. for 5 min.

Fig. 3. Sanctity, treated 13 November; 130° F. for 1 min.

(*Received* 13 *June* 1949)

Fig. 1

Fig. 2

STANILAND—*Experiments on the control of chrysanthemum eelworm*

Fig. 1

Fig. 2

Fig. 3

STANILAND—*Experiments on the control of chrysanthemum eelworm*

VERNALIZATION OF WINTER RYE BY ULTRASONICS

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Petkus winter rye seed was subjected to ultrasonic vibrations of 1 Mcyc./sec. frequency in order to test the possibility of their having a vernalizing action. Seed which had not been soaked, fully imbibed seed, germinating seed (radicle showing) and seedlings with about 2 mm. long coleoptiles were treated with two intensities of ultrasonics for two periods of exposure.

There is a slight favourable effect on germination rate of the unsoaked seed, but injury or death is caused by higher doses applied to the imbibed or germinated seed. Time to flowering was not affected by this treatment and no vernalization resulted.

The effects of ultrasonics on biological and other materials have recently been summarized by Parthasarathy & Pande (1945). Among the many effects cited, the authors mention claims by Russian workers to have vernalized winter cereals by ultrasonic vibrations. Unfortunately, no direct reference is given in this paper, so that details of method and results of this Russian work could not be traced. In view of the practical advantages which might be gained it appeared worth while attempting to repeat these experiments. The chief difficulties of low-temperature vernalization are due to the long period of cold storage required, and to the use of partly germinated grain, with attendant complications of fungal infection, etc. These would be avoided by ultrasonic treatment.

Apart from this Russian work on cereals, data are given by Martinec (1943) on the effect of ultrasonics on millet seed and seedlings. He measured plant heights and dry weights 28 days after treatment, but no data are given relating to effects on time of flowering.

Methods

The ultrasonic apparatus consisted of a high-frequency oscillator connected to a quartz crystal which vibrated at a frequency of 1 Mcyc./sec. The crystal and holder were immersed in an oil-bath, through which the vibrations were transmitted to the object under treatment.

The vessel containing the seeds was made of $1\frac{1}{2}$ in. glass tubing with a cellophane membrane fixed over the bottom. It was placed in the oil-bath $1\frac{1}{2}$ in. above the crystal surface. The batch of twenty seeds was placed in water in the container, and a cooling coil was immersed in the water. The temperature of the water was kept below 32° C. by this device.

Power output was adjusted by variation of the voltage across the crystal. In this experiment voltages of 4500 and 3000 were used, to give the two levels *A* and *B*, of

acoustic energy. Measurements of temperature rise in tap water in the container gave values of 48 and 27 W. for A and B. There was, however, no estimation of the power absorbed by the seeds themselves.

The experiment was designed factorially to cover a wide range of conditions: two energy levels of ultrasound (A, B), applied at four germination stages of the seed (1–4) for two periods of exposure (T_1, T_2). The stages of germination were: (1) dry seed, (2) fully imbibed seed before germination, (3) germinating seed, radicle emerged, and (4) germinated seed, coleoptile about 2 mm. long. Under the conditions of the experiment stages 2, 3 and 4 were reached 6, 14 and 38 hr. after soaking. Petkus winter rye seed was used, and was kindly supplied by Dr O. N. Purvis. The seed was soaked for 2 hr. and then placed on moist filter-paper in an incubator at 22° C. until treated; it was planted the day after treatment. The period of exposure was 10 or 20 min., a control group being left untreated in each case.

The seeds were planted in large glazed flower-pots, each receiving six plants, which were later thinned to three. Three pots, containing in all nine replicate plants, were grown for each treatment. As the factorial design involved one dummy treatment the control set included twice this number of replicates.

Later a further batch of seed was treated and sown in open ground. Here there was only one treatment besides the controls, i.e. exposure to the higher intensity vibrations for 10 min., the seed having been soaked for 24 hr.

Results

The treatment effects observed may be divided into two groups: effects on germination, and effects on flowering behaviour.

Effects on germination

Two distinct effects on germination were noted. The germination rate of the unsoaked seed was slightly accelerated by the treatment, as shown in Table 1.

Table 1. *Percentage germination of unsoaked seed 1 week after sowing*

	Germinated	Not germinated	
Untreated	27 (75 %)	9 (25 %)	$\chi_1^2 = 5{\cdot}18$
All ultrasonic treatments	66 (92 %)	6 (8 %)	

For $P = 0{\cdot}05$, $\chi_1^2 = 3{\cdot}84$; $P = 0{\cdot}02$, $\chi_1^2 = 5{\cdot}41$.

In contrast to this, treatment of seeds sprouted or imbibed prior to exposure markedly reduced germination. Thus the final percentage germination is very significantly higher for the untreated seed (Table 2).

Further analysis of the germination data of the treated seeds shows highly significant differences between the two times of exposure and the two energy levels of the

vibrations (Table 3). Finally, the χ^2 for the interaction between stage of germination and energy level also exceeds the $P=0{\cdot}001$ probability level (Table 4).

TABLE 2. *Final percentage germination of treated and untreated seed*

	Germinated	Not germinated	
Untreated	136 (94 %)	8 (6 %)	$\chi_1^2=16{\cdot}9$
Treated	228 (79 %)	60 (21 %)	

For $P=0{\cdot}001$, $\chi_1^2=10{\cdot}8$.

TABLE 3. *Effects of time of exposure and acoustic energy on final percentage germination*

	Germinated	Not germinated	
T_1 (sum of A and B)	126 (88 %)	18 (12 %)	
T_2 (sum of A and B)	102 (71 %)	42 (29 %)	$\chi_1^2=12{\cdot}1$
A (sum of T_1 and T_2)	135 (94 %)	9 (6 %)	$\chi_1^2=37{\cdot}1$
B (sum of T_1 and T_2)	93 (65 %)	51 (35 %)	

For $P=0{\cdot}001$, $\chi_1^2=10{\cdot}8$.

TABLE 4. *Interaction effects of acoustic energy and stage of germination on final percentage germination*

Stage of germination of seed when treated ...		1	2	3	4
Acoustic energy:	A	35 (97 %)	34 (94 %)	33 (92 %)	33 (92 %)
	B	35 (97 %)	26 (72 %)	21 (58 %)	11 (31 %)

$\chi_3^2=21{\cdot}1$.
For $P=0{\cdot}001$, $\chi_3^2=16{\cdot}2$.

It will be seen from Tables 3 and 4 that the injurious effect of the vibrations is largely confined to the higher energy level, and particularly to the partly germinated seed; prolonged exposure enhances the effect.

Germination of the second batch of seed grown in the open ground was even more depressed by the treatment, being below 15 % as against 83 % in the untreated.

Effects on flowering behaviour

Elongation of the main stem and principal tillers, i.e. 'shooting' is the first macroscopic sign in cereals of the change-over to the reproductive phase. Once this process has been initiated flower development will run its normal course. The time taken for the first signs of shooting to appear was therefore chosen as a measure of any treatment effects on flowering behaviour.

Apart from a single rogue plant (spring rye seed), shooting did not begin until 85 days from sowing. After this time increasing numbers of plants scattered irregularly over all treatments began to shoot. No differences could be detected between treated and untreated plants. Generally, their flowering behaviour was exactly that to be expected of unvernalized winter rye plants subject to a few cold

nights only. The percentages of treated and untreated plants which were beginning to shoot at various dates after sowing are shown in Table 5.

Table 5. *Percentage of plants shooting at various dates after sowing*

Days after sowing	Treated (%)	Untreated (%)
85	4·7	5·8
90	5·5	7·2
100	40·9	39·1
109	59·1	50·7
123	78·7	73·9

Discussion

These results show clearly that exposing winter rye seed, dry or germinated, to ultrasonic vibrations of this frequency does not reduce the time to flowering, and that there is no 'vernalizing' action. From the effects on germination itself it appears that the treatment range chosen was wide enough to embrace anything from slightly favourable to lethal conditions, and it is therefore unlikely that other treatment levels, at this frequency, would give different results.

It seems probable that the slightly increased germination rate following treatment of the unsoaked seed was due to acceleration of the rate of entry of moisture. Water uptake was measured as a percentage of dry weight for treated and untreated seeds, in water at the same temperature for the same length of time, and the figures obtained were 28 and 11% respectively.

Injurious effects were confined to the imbibed or germinated seed, their severity increasing with the degree of sprouting. Some evidence was found that the coleoptile and probably also the root-tip were particularly sensitive, for, apart from the seedlings actually killed, seven other seedlings were found whose coleoptile had been injured and whose first leaf was emerging below the soil surface. All these seedlings had been soaked or sprouted before treatment, and had been exposed to the higher intensity of ultrasonic vibrations. According to Martinec (1943), mechanical damage may be caused by the high-pressure gradients developed. In the present case, there is also the possibility of injury by internal heating. The fact that seed not previously soaked remained uninjured may possibly be due to layers of air enclosed by the testa, and serving to reflect the vibrations.

Our thanks are due to Mullard Radio Valve Co. for apparatus which was placed at our disposal.

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(*Received* 19 *May* 1949)

STUDIES OF THE CLOVE TREE

2. HISTOLOGY, WITH SPECIAL REFERENCE TO SUDDEN DEATH DISEASE

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(With Plates 3, 4 and 5 and 4 Text-figures)

A detailed study was made of the histology of apparently healthy clove trees and of trees expected to die from sudden death disease, in an attempt to find either a diagnostic character of the disease or some indication of a possible pathogen.

The apparently healthy clove tree differs from a typical dicotyledon in a number of ways. This is especially true of the vascular system where, not only are the bundles bicollateral, but, in the stem, secondary thickening occurs in the *internal* phloem, necrosis of the first formed cells keeping pace with the production of new cells. A definite cambium is found only to the exterior of the xylem and this is exceptionally narrow and the cells have very delicate walls. Phloem necrosis may occur in any part of the tree. The development and lignification of the xylem is very uneven, but this seems to bear no relation to the season. Tyloses are formed in the vessels of the root and, to a less extent, in those of the stem. Starch reserves are usually small, but large quantities of tannins, oils and calcium oxalate are secreted in all tissues. The oil may be contained in special schizogenous glands which are abundant in the aerial parts of the tree. Much mechanical tissue, consisting of fibres and stone cells, is formed in the pith and cortex of the stem and in the phloem in all parts of the tree, but lignification of this tissue is very uneven. Occasional dead cells are found in all tissues, even in the primary meristem. In the leaf, the plastids develop very unevenly; this is followed by retarded development of the pigment in some parts, resulting in a flecked appearance. Stomata are exceptionally numerous; in the leaf, they are confined to the lower surface and always communicate with very small cavities. Air spaces in the mesophyll are very small and are almost non-existent in other tissues. Bodies, morphologically similar to the amorphous intracellular inclusions due to some viruses but differing chemically from them, are abundant in the leaves.

Some of these characters are usually associated with a diseased condition, but they occur in both healthy and diseased cloves in the Zanzibar Protectorate. In the sudden death suspect, some may occur earlier or be more accentuated than in the healthy tree. This evidence alone might suggest that sudden death is merely the culmination of a long period of adverse conditions, but other evidence suggests very strongly that the disease is due to a pathogen. Of the groups of organisms which may cause disease, only fungi were found. These occurred in the absorbing roots but are unlikely to be the pathogens causing sudden death, for, until the tree has actually wilted, very few hyphae are present, and no more than are found in healthy trees. Nothing incompatible with the suggestion that sudden death is caused by a virus was found.

* Seconded from Rothamsted Experimental Station, Harpenden, England.

Introduction

Most of the World's supply of cloves is produced in the plantations of the Protectorate of Zanzibar, whose economy is largely dependent on this industry. Interest has recently been focused on the clove tree, *Eugenia aromatica* (Baill.), because these plantations are being devastated by a disease which has been called 'sudden death'. Although many suggestions have been made as to its cause, none has yet been proved and it seemed possible that a detailed microscopical examination might either give a clue as to its origin or reveal some early diagnostic symptom. Apart from some work by Campbell in 1938–9, little seems to have been done on the anatomy of the clove since Lignier's work towards the end of the nineteenth century. Lignier's original papers were not available and, as Campbell's work was directed primarily to discovering whether or not sudden death was of fungal origin, his anatomical studies were not very detailed. It was necessary, therefore, to make a detailed study of the histology of both healthy and diseased trees.

Material and methods

The external symptoms of the disease have been fully described elsewhere (Nutman & Sheffield, 1949) when it was explained that the name, sudden death, is probably due to the fact that the final stages of the disease are an excessive leaf-fall followed by a rapid wilt of the remaining leaves which remain adhering to the tree until removed by wind or rain. It was also explained that the name is misleading for, in a stand of good trees, a trained observer can often diagnose the disease months before death occurs. Concurrently with this work, considerable circumstantial evidence was accumulating to suggest that sudden death is caused by a pathogen. In view of this deduction, the obtaining of control material in islands where the disease is so widespread presented a major problem. In Pemba, cloves cannot be found more than $\frac{1}{4}$ mile from sudden death areas, but there are a few to the north of Zanzibar Island which are about 5 miles from trees known to be affected with sudden death. Control material was collected from these and seedlings from the Government nurseries were also used. Material was examined from trees expected to die within weeks or months, from trees just before the wilt set in and, in order to bring this work into line with that of Welsford and that of Campbell, from already wilted trees. A year after control material was collected, no sudden death could be found in the district from which the material was obtained, and a check was also made to see that the suspect trees chosen did die in the typical sudden death manner.

Some material from Pemba was examined but facilities and transport difficulties confined most of the histological work to Zanzibar. Most of the diseased material was collected from a plantation, 12 miles from the laboratory, where sudden death is now very active and the Northern plantation used for the collection of healthy material is 25 miles from the laboratory. These distances are important as they were to some extent a limiting factor in the techniques used, for, although all results were

checked on living material, it was necessary to use fixed material more often than was desirable.

Samples were taken from all parts of the trees. Living stems and leaves were brought to the laboratories by cutting a large branch and, at once, putting the cut end in water. Roots were either immersed in water or wrapped to avoid excessive loss of moisture. Living material was examined on the day of collection.

Before fixing, roots and stems were cut into $\frac{1}{2}$ in. lengths and blocks were cut from the trunk, collar and larger roots. Six to eight pairs of leaves are usually found on each twig. The top ones are pink but, when about an inch long, they become yellow, then flecked with green and finally dark green all over. In the healthy tree, they lose their green colour, becoming bright green just before abscission. In a late stage of the disease, it may be impossible to find any green leaves on the tree: all are yellow but, in other cases, the leaves absciss whilst still dark green. For fixing, the leaves were divided into four groups: less than 1 in. long; young, yellow; flecked and oldest (green or bright yellow). Leaves were cut into pieces about $\frac{1}{2} \times \frac{1}{4}$ in. immediately before fixing.

All fixing was done in the field and, where appropriate, an exhaust pump was used. After some trials, the following fixatives were chosen:

Tissue	Fixative
Leaves	Allen's modification of Bouin's fluid
Growing points of stems	Champy's fluid (after 1 min. immersion in Carnoy's fluid)
Stems, Trunk, Collar	Formalin acetic acid alcohol Formalin propionic acid alcohol
Roots (absorbing)	Nawaschin's fluid Champy (after 1 min. in Carnoy)
Roots (older)	Formalin acetic acid alcohol Formalin propionic acid alcohol

Softer tissues (either fixed or fresh) were cut by hand or after freezing in gum syrup; and harder tissues, by sledge microtome. Some of the softer tissues were dehydrated and then cleared in cedar-wood oil where they were stored for a time. Later these were embedded in paraffin wax of m.p. 60° C., preparatory to sectioning.

The stains chosen were designed to show the anatomical and cytological features, possible pathogens and those abnormalities sometimes associated with virus infection. They included: crystal violet (with and without mordanting and differentiating in iodine), safranin, Feulgen's reagent, Heidenhain's haematoxylin, all with appropriate counter-stains such as fast green, erythrosin, orange G, and Bismarck brown; carbol-thionin differentiated in orange G; cotton blue; phloroglucinol, etc.

The shoot

The stem. In both healthy and diseased trees, the primordial meristem of the shoot is that typical of dicotyledons except that, even in the healthy tree, a few of the

young cells, especially in the epidermal layer, may die and then become flattened by the growth of neighbouring cells and absorb all dyes readily. The leaf primordia are wrapped around the stem apex. One or two buds are formed in the axil of each leaf, but often one of these dies at an early stage (Pl. 3, fig. 1).

The primary vascular strand is marked out very near the stem apex and differentiation of the vascular tissues occurs very soon. Above the first pair of leaves recognizable by the naked eye, the stem consists chiefly of closely packed parenchymatous cells and contains a ring of twenty to thirty vascular bundles. The bundles are bicollateral and each consists of two to three xylem elements bounded both to exterior and interior by groups of about six phloem cells. Between the xylem and the external phloem, a layer of cambium can usually be distinguished, but no cambial tissue can be recognized between the xylem and the internal phloem. The pith is parenchymatous and is about twelve cells in diameter and the cortex is about eight to twelve cells wide. At this stage both these tissues are formed of thin-walled parenchymatous cells with a few very small air-spaces between those of the cortex but scarcely any between those of the pith. Chloroplasts are found in the cortex and a ring of developing oil glands lies immediately below the thick-walled epidermis (Text-fig. 1 *a*).

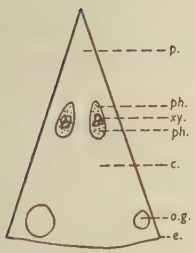
Secondary thickening soon commences (Text-fig. 1 *b*, *c*). The cambium forms a complete ring and xylem cells are cut off to the inside and phloem cells to the outside of it. No internal cambial ring could be distinguished, but the new phloem cells are continually formed in that part of the internal phloem nearest the primary xylem. Solereder (1908) states that when intraxylary phloem occurs in the Myrtaceae it is often supported by groups of bast fibres. In the clove, very soon after secondary thickening commences, fibres are differentiated in both primary internal and primary external phloem.

At about the seventh node from the tip of the shoot, obliteration of the inner part of the internal phloem and the outer part of the external phloem commences in the healthy plant. In a sudden death suspect this obliteration usually starts rather earlier. Further elements continue to be produced in the cambium outside the wood and from pre-existing elements inside the wood. Xylem elements also continue to be cut off to the inside of the cambium but, as will be seen later, it is seldom possible to distinguish annual rings.

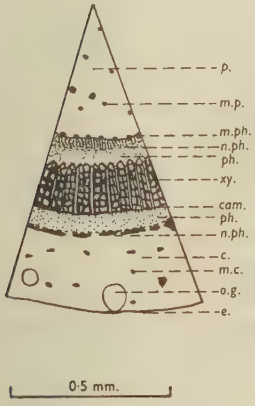
The vascular tissue is crossed by numerous medullary rays, usually two cells only in width.

Simultaneously with the secondary growth in thickness, mechanical tissues are formed. A number of the rectangular parenchymatous cells of the pith develop into thick-walled stone cells. Commencing immediately below the epidermis, the cortex becomes first collenchymatous and later develops into thick-walled sclerenchymatous tissue, which finally becomes continuous with the fibres of the older phloem cells. During this process the oil glands may become crushed. Tegumentary tissues are also developing, the epidermis becomes suberized and a cambium is formed

immediately below it, which cuts off cork cells towards the exterior until a many-layered phellogen is formed which may crack on the exterior. Lenticels are formed in the cork.



Text-fig. 1 *a*.



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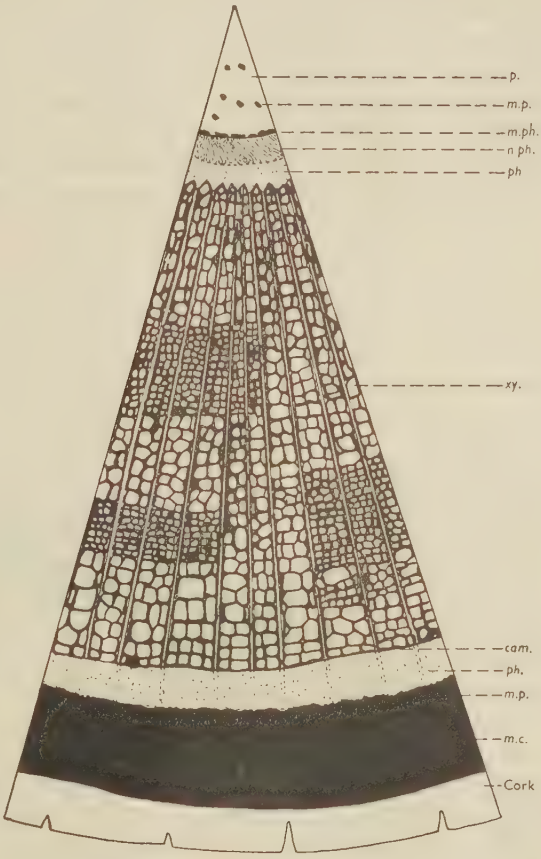


Text-fig. 1 *c*.

Text-fig. 1. Transverse sections (*a*) of young stem; (*b*) of stem soon after commencement of secondary thickening; and (*c*) of older stem. *p.* pith; *m.p.* mechanical tissue in pith; *ph.* phloem which is mainly functional but, in (*b*) and (*c*), contains some necrotic groups; *m.ph.* mechanical tissue in phloem; *n.ph.* necrotic phloem; *xy.* xylem; *cam.* cambium; *c.* cortex; *m.c.* mechanical tissue in cortex; *o.g.* oil gland; *e.* epidermis.

Growth in thickness continues throughout the life of the plant, but increase in girth is due almost entirely to increase in the amount of xylem. In a tree about $\frac{1}{2}$ m. in diameter, only about 1 mm. is due to the cork and 5–20 mm. to the external phloem. One-half to three-quarters of the width of the phloem consists of obli-

terated cells and fibres. The internal phloem is obliterated soon after formation, but a few of the newest formed cells are always functional. Blocks cut from the collar of the tree showed no essential differences from the trunk.

The Leaf. The leaf primordium at first consists of cells similar to those of the apical meristem, but cell division soon becomes limited to the apices of the primordia (Pl. 3, fig. 1). Soon the primary meristem is differentiated and cell division in the outer tissues ceases, except in specialized regions, and growth occurs only by increase in cell size. When about $\frac{1}{2}$ in. long, the leaf lamina in cross-section consists of seven to nine layers of closely packed rectangular cells, surrounded by a smaller celled epidermis, with a rapidly thickening cuticle. From some cells of this, oil glands and stomata now begin to develop (Pl. 3, fig. 3).

The midrib at this stage consists of a number of more or less isolated vessels, usually slightly lignified, scattered more or less in the shape of a 'U' (Text-fig. 2*a*; Pl. 3, fig. 4). Small strands of phloem, each consisting of four to six cells, are found both above and below the xylem elements. The parenchymatous cells which surround the vascular bundles are isodiametric and rather larger than are those forming the main part of the leaf blade. Some of these cells, as well as some of those which should later form the assimilating tissue, may even at this stage be necrotic, even in a leaf from a healthy tree.

As the leaf increases in size, the two top layers of parenchymatous cells develop into palisade tissue. The uppermost layer elongates until the cells are about three times as long as wide and in the second layer about twice as long. Occasional dark-staining dead cells are found in the palisade tissues of both healthy and diseased plants. Increase in size of the lower layers of cells is approximately equal in all directions. They continue to adhere closely together so that air spaces formed in the spongy mesophyll are very few and unusually small (Pl. 3, fig. 5).

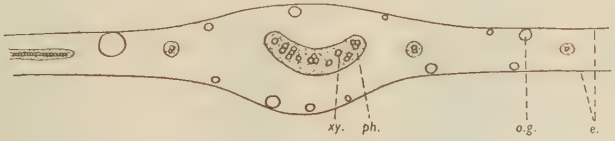
Simultaneously with this increase in size, the chloroplasts develop. Their formation is uneven, those nearest the veins usually developing first. Sometimes leaves pass gradually from a pale to a dark green colour but more often the colour develops unevenly, giving the leaf a mottled appearance, with yellow flecks between the green areas surrounding the veins. This flecking may occur in both healthy and diseased plants, and is due to unequal development of the pigment, a reflexion of the retarded development of the plastids in some parts of the leaf.

Leaves usually become dark green in both healthy trees and in trees already suspected of having the disease. In the healthy tree, just before abscission, the chloroplasts disintegrate, and as a result the leaf assumes a bright yellow colour. In a tree which dies rapidly with sudden death disease, some leaves may absciss while still green but more often they gradually lose their green colour so that a 'certain suspect' is often characterized by the large number of yellow leaves adhering to it. This yellowing is due to destruction of the plastids and it differs from that of the normal tree only in that it occurs in much younger leaves (Pl. 3, fig. 8).

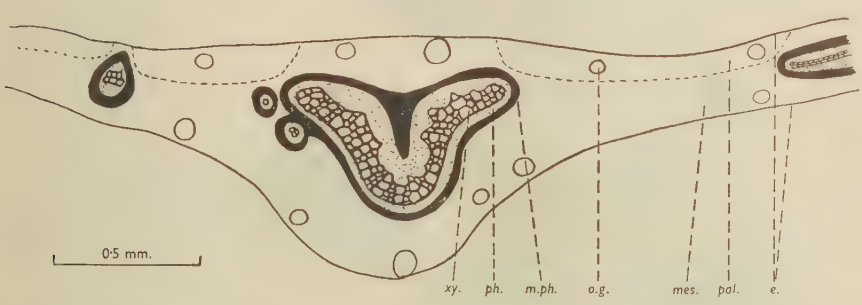
The midrib of the mature leaf has a very characteristic form which develops from

the former isolated groups of xylem and phloem cells by continual division and differentiation until a continuous band is formed. This band is in the form of a 'U' with the two ends bent outwards. As the bundles are bicollateral, the xylem of the midrib is completely surrounded by phloem. The outer phloem cells develop into fibres until the whole bundle is completely enclosed in a ring of sclerenchymatous tissue (Text-fig. 2*b*; Pl. 3, fig. 6). At a late stage, phloem obliteration and necrosis may occur in both healthy and diseased trees. This will be considered later.

The side veins of the leaf are oval in transverse section but their structure is essentially similar to that of the midrib. Each originates from a single bicollateral bundle and consists of a group of vessels and tracheids with phloem below it and



Text-fig. 2*a*.



Text-fig. 2*b*.

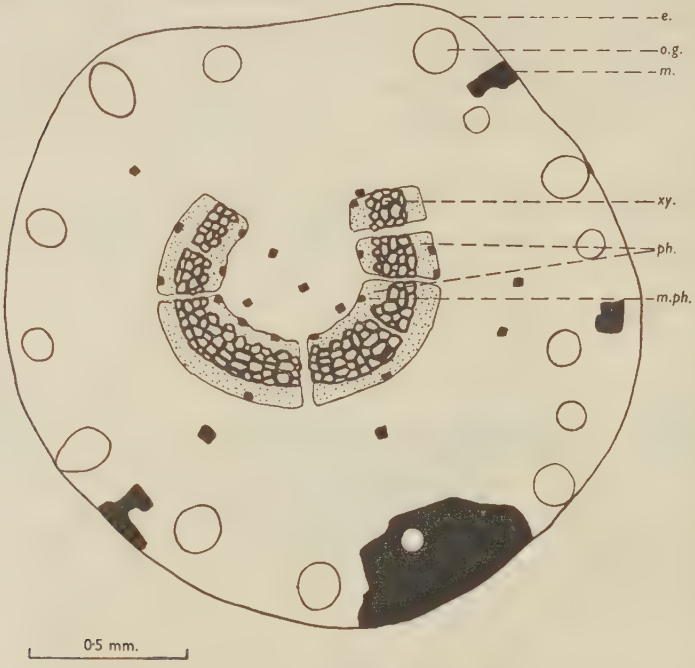
Text-fig. 2. Transverse sections (*a*) of young leaf, and (*b*) of mature leaf. *e.* epidermis; *pal.* palisade tissue; *mes.* mesophyll; *o.g.* oil gland; *ph.* phloem; functional but in (*b*) containing some necrotic and some blocked cells; *m.ph.* mechanical tissue in phloem; *xy.* xylem.

a rather smaller group of phloem above. Fibres are differentiated in the phloem until the vascular strand comes to be surrounded with a double layer of sclerenchymatous tissue (Text-fig. 2*b*; Pl. 3, fig. 5).

In some species of *Eugenia*, the epidermal cells have been described as excessively large (Holteman, quoted by Solereder, 1908), but in the clove the reverse is the case (Pl. 3, fig. 5; Pl. 5, fig. 2). Stomata occur on the lower surface only of the leaf (Pl. 5, fig. 2), but oil glands are very abundant in the leaves and occur towards both surfaces (Pl. 3, fig. 3).

The petiole is characteristically cylindrical but flattened on the upper side. The vascular system is 'U'-shaped and is essentially similar in structure and develop-

ment to that of the midrib, although its development is always further advanced. The ground tissue consists at first of isodiametric parenchymatous cells bounded by a strongly cuticularized epidermis. Later stone cells may form in the pith, phloem and cortex. In any transverse section, a ring of twenty to thirty oil glands is found directly below the epidermis (Text-fig. 3).

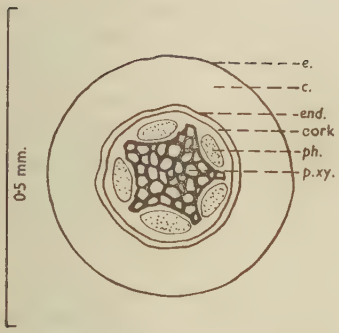


Text-fig. 3. Transverse section of petiole of mature leaf. *e.* epidermis; *o.g.* oil gland; *m.* stone cells; *xy.* xylem; *ph.* phloem, partly functional but containing blocked and necrotic cells; *m.ph.* phloem fibres.

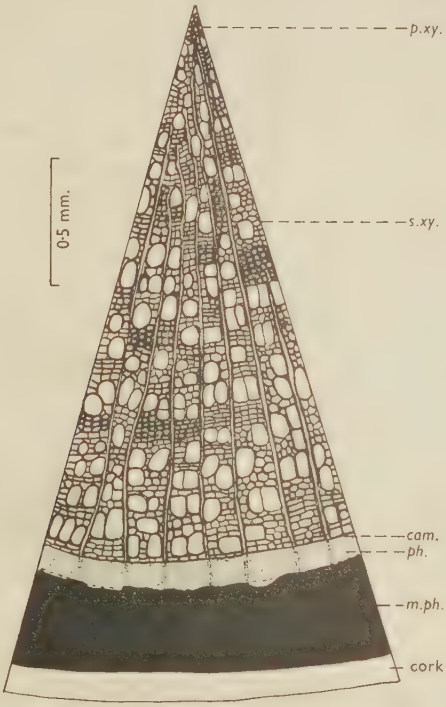
THE ROOT

In the healthy clove a dense mass of fibrous roots lies closely underneath the surface of the soil. Their absorbing tips vary in size and number at different seasons of the year. In the early part of 1948 it was difficult to find any in this surface layer and those found were never more than 0·5 mm. in diameter. After the cessation in June of the long rains brought by the south-west monsoon, the roots increased in size, and by October it was sometimes possible to find tips as much as 3 mm. in diameter. These absorbing tips are ephemeral structures even in a healthy tree and at all seasons may blacken prior to dying back. When a tree first becomes suspected of sudden death, its absorbing roots are invariably found to be reduced in number and, as the disease progresses, become increasingly difficult to find, although the main

laterals appear to be unaffected. Campbell (1940) found stunted absorbing roots with an abnormal degree of branching on sudden death trees and they usually had fungi both outside and invading the roots. I have found a few similar roots but they were smaller than those figured by Campbell and occurred also on healthy trees. It is possible that they are formed by the dying back of the main tip and the consequent production of laterals.



Text-fig. 4*a*.



Text-fig. 4*b*.

Text-fig. 4. Transverse sections (*a*) of young root immediately before secondary thickening commences, and (*b*) of older root. *p.xy.* primary xylem; *s.xy.* secondary xylem; *cam.* cambium; *ph.* phloem; mainly functional but in (*b*) with some necrotic groups and blocked cells; *m.ph.* phloem which has become mechanical in function; *end.* endodermis; *c.* cortex; *e.* epidermis.

It seemed possible that an examination of the absorbing roots would give some clue as to the cause of the disease, but neither macro- nor microscopic examination revealed any appreciable difference between those of healthy and suspect trees. Those roots which were still active seemed sometimes to be shorter in the suspect, but otherwise no difference could be found. A few fungi were found in those roots which died back, in healthy and suspect trees alike.

A longitudinal section of the root-tip shows it to be protected by a root cap which

arises from a special meristem layer (Pl. 3, fig. 2). This cap covers and surrounds the primordial meristem. The epidermis near the tip is fairly thin-walled and, over a certain distance varying with the season and the size and growth of the absorbing roots, root hairs are found but are never very abundant in the clove. Glover (1939) has stated that in the roots of sudden death trees, the endodermis is almost completely suberized to within 0·5 mm. of the root tip. This I was not able to confirm. Most of the root tissue contains tannin and it is especially concentrated in the root cap, the epidermis and the endodermis. In unstained preparations it imparts a red colour to the cells, making it impossible to test for suberin with the usual reagent such as Sudan III and Sudan IV.

When differentiation of the tissues begins, the young root is seen to be occupied by about six groups of protoxylem alternating with an equal number of strands of phloem. The whole is surrounded by a pericycle and by an endodermis, which is thickened on the inner tangential and on the radial walls, and a thin-walled cortex bounded by an epidermis lies outside these (Text-fig. 4*a*; Pl. 5, fig. 5).

A ring of cambial tissue becomes marked out between the protoxylem and the phloem and produces secondary vascular tissues in the usual way. As in the stem, there is no regular formation of annual rings, many narrow medullary rays cross the vascular system and fibres are differentiated in the first formed phloem. Just before secondary thickening commences, the pericycle becomes meristematic and produces cork cells to the exterior and all tissues outside this layer slough off (Pl. 5, fig. 5). As secondary thickening goes on, almost all the outer phloem cells become sclerenchymatous; a few are fibrous, but the majority develop into stone cells (Pl. 4, fig. 9). Obliteration and necrosis of the phloem occur as in the stem, so that in an old root, only the newest formed cells are functional (Text-fig. 4*b*).

Meristems

The primary meristem of the shoot and root and of the leaf primordia all consist of small closely packed cubical cells with no air spaces. These cells contain very large nuclei and dense cytoplasm. Just behind the growing point, the three layers, dermatogen, periblem and plerome soon become differentiated. The plerome consists of a central cylinder of roughly cubical cells and is surrounded by longitudinal strands of rapidly elongating cells which divide only longitudinally. These strands also pass into the bases of the developing leaf primordia (Pl. 3, figs. 1, 2). The cells of the strands now differentiate into protoxylem and protophloem and the cells of the central cylinder form the pith. The latter enlarge but remain rectangular with no air spaces throughout the life of the plant (Pl. 4, fig. 10).

Between the xylem and the phloem two layers of cells remain meristematic and become the interfasicular cambium. This cambium is similar in structure and behaviour to that of the typical dicotyledon, except in that the cell walls are exceptionally delicate, and this, combined with the large amount of mechanical tissue in the

clove, makes it extremely difficult to obtain sections which do not break at the cambial layer (Pl. 3, figs. 9, 10).

According to Solereder (1908), the place of origin of the cork is of value in classification of members of the Myrtaceae, and, without mentioning whether he is referring to stem or root, he says it may arise immediately subepidermally, from a deeply seated layer of the primary cortical parenchyma or from the pericycle. In the clove, the origin is different in stem and root. In the stem, a layer of cells immediately below the epidermis becomes meristematic and cuts off cells to the outside. In the root, the layer of cells immediately within the endodermis assumes a meristematic function. This cuts off cells to the exterior and when these become suberized, water is cut off from the cortical and epidermal cells which soon dry up and flake off (Pl. 5, fig. 5).

Xylem

In all parts of the plant, it is characteristic of the xylem that it contains a high proportion of tracheids, relatively few vessels and very little parenchyma. The vessels when first formed are spirally thickened but rapidly pass through the reticulate to the pitted stage. Each individual vessel or tracheid is usually fairly evenly thickened over its whole surface, but there is great variation in the thickening of different cells. Lignification of both vessels and tracheids is very uneven even in a single cell. A single cell stained in a lignin dye often appears in different shades of colour over its apparently evenly thickened surface. In comparable parts of the same section both vessels and tracheids may be thick-walled and strongly lignified; they may be thin-walled and lignified or either thin- or thick-walled and almost or quite unlignified (Pl. 4, fig. 1). Groups of adjacent cells are usually similarly thickened but it was not possible to correlate the variation with any other character. Cells at equal distances from the centre of the stem or root, and presumably of simultaneous formation, are often of different sizes and quite different in thickening and lignification. As there seems to be no regular variation in cell size with changing season, it was usually quite impossible to recognize annual rings. Thickening is usually less marked in the leaf than in other tissues (Pl. 4, fig. 4).

As the xylem increases in age, the vessels often become blocked with a brown granular substance. This blocking occurs in both healthy and diseased tissues in all parts of the tree, but usually begins sooner in the suspect tree. For example, it begins in the healthy leaf about the time it is fully green but, in the suspect plant, a large proportion of the vessels may be blocked even before the leaf has reached the flecked stage (Pl. 4, fig. 4). This agrees with the findings of Glover (1939) and is possibly, as he suggests, the explanation of the reduced transpiration rate in suspect trees. He quotes Campbell as saying that this blocking shows a seasonal variation in intensity in healthy trees. This I have not observed.

Tyloses may be formed in the vessels of the stem and more particularly in those of the roots. The tyloses are formed by intrusion into the vessels from the paren-

chymatous cells which adjoin them. Often a large number of tyloses intrude into a single vessel forming a vertical row which entirely blocks it (Pl. 4, figs. 2, 3). Heim & Bouriquet (1937, 1939) and also Campbell (1940) found no tyloses in the vessels of healthy trees, but the former found them in cloves dying of 'apoplexie', which is probably synonymous with sudden death, and the latter found them in sudden death trees. I have found that, although they are more prevalent and present in greater numbers in suspect than in healthy trees, they can invariably be found in the latter if sufficient search be made.

Phloem

In all the aerial organs of the clove, the vascular bundles are bicollateral, having phloem elements both to the inside and outside of the xylem (Pl. 3, fig. 4). In a young organ, each phloem strand consists of a few sieve tubes and companion cells and a little parenchyma. The sieve tubes have latticed side walls and slightly oblique end walls. The companion cells have large nuclei and dense cell contents. There seem to be no air-spaces in the phloem tissues. No abnormal inclusions were found in the phloem cells.

The phloem strands to the interior of the xylem are usually smaller than those to the exterior. In the stem the external phloem is separated from the xylem by two or three layers of cambial cells, which divide to produce new phloem and xylem tissues (Pl. 3, figs. 9, 10). The internal phloem also increases in quantity as the stem grows in thickness. Secondary thickening of the internal phloem is very unusual: it does not characterize the genus *Eugenia*, but Solereder (1908) quotes Petersen as having found it in *E. Michellii* (syn. *uniflora*). In the clove no definite cambial layer could be found and the phloem presumably increases by division of previously existing cells. The isolated strands join to form a complete ring which then increases in width, the increase taking place on the side nearest the xylem (Pl. 4, fig. 4). As the xylem forms an inelastic cylinder, this increase in the internal phloem obviously cannot continue unless some compensating destruction of the tissues occurs towards the centre of the stem. The first formed phloem therefore soon becomes obliterated and then necrotic, taking a deep stain with phloroglucinol. Increase in the width of this necrotic ring keeps pace with the production of new cells near the xylem. Necrosis is often associated with division of the adjoining cells, but in tea (Bond, 1947) and in potato infected with leaf roll virus (Sheffield, 1943), as the necrotic phloem cells collapse, the adjoining ones tend to increase in size. This may happen also even in the internal phloem of the clove where there is very little room for expansion. Those cells which do not necrose lie usually, but not invariably, on the same radii, so that the most internal phloem comes to consist of wide radiating bands of necrotic cells alternating with single rows of enlarged but still living cells. The very small cavities sometimes left in these necrotic cells often become blocked with a brown granular substance (Pl. 4, figs. 7, 8).

In the outer phloem, lignified fibres are very soon differentiated. Some cells

become blocked with brownish contents, whilst others are completely obliterated and obliteration may be followed by necrosis (Pl. 4, fig. 6). Still others are converted into very thick-walled stone cells (Pl. 4, fig. 9). As new cells are produced by cambial activity, more and more cells to the outside either assume a mechanical function or become completely non-functional, so that in an old stem only a very small proportion of the phloem functions for conduction. This is the latest formed phloem and amongst it scattered groups of obliterated or necrotic cells may be found.

In the leaf lamina and petiole, the structure of the first formed phloem is similar to that in the stem. As in the stem, the isolated strands join up and they form a single U-shaped stele. At the same time fibres are differentiated in the outer part of both internal and external phloem until a layer, 1 to 3 cells wide, completely encloses the stele. As in the stem, blocking and obliteration and necrosis of some of the cells occur (Pl. 3, figs. 6, 7).

In the root the development of the phloem follows much the same course as that of the external phloem of the stem.

It is generally true to say that the sieve tubes of any plant cease to function after one season. After that they may remain open or be obliterated. Although it does occasionally occur in healthy plants (e.g. tea) phloem necrosis is usually the result of a diseased condition (cf. p. 41).

In the clove, necrotic cells seem to be scattered at random, in all organs. The only differences that could be found between the healthy and diseased tissues were in the time of onset and degree of blocking, obliteration and necrosis: as with blocking of the xylem, degeneration of the phloem starts some time after the healthy leaf has become fully green, but in a suspect tree it may be well advanced by the time the leaf has reached the flecked stage.

In one plantation where sudden death is spreading rapidly, the walls of the phloem cells of the roots were sometimes discoloured, appearing bright yellow. Living roots of about $\frac{1}{4}$ in. diameter from a number of trees were sectioned and examined fresh without treatment. The phloem of apparently healthy trees was free from the discoloration, whilst that from trees in an advanced stage of the disease was entirely yellow. All intermediate conditions were found and the amount of discoloration was an index of the amount of decline. This was so definite that sections from about a hundred roots were correctly diagnosed even to the degree of decline of the tree from which they were taken. However, when roots were taken from healthy trees from three districts, all some distance from sudden death areas, they sometimes had discoloured patches in their phloem. The only factor common to the three healthy plantations and differing from the diseased one was the nature of the soil. The latter was on a light sandy soil, whilst the former were on the more usual red coralline soil. It is possible that the discoloration can be produced by either of two causes: by sudden death disease or by a soil factor. This is impossible to check as there are no healthy plantations in the light sandy soil.

Medullary rays

According to Solereder (1908), narrow medullary rays are characteristic of the natural order, Myrtaceae. In the clove, in both root and stem they are very numerous and are usually only two cells in width in a horizontal plane. They are of interest chiefly because of the very distinct pits that can be seen in their walls. The cells are often filled with tannin which helps to show very clearly the connexions between the protoplasts of adjoining cells (Pl. 5, fig. 1).

Mechanical tissues

Large amounts of mechanical tissue are formed in many parts of the clove and most of these have already been referred to under other headings. Comment was made on the high proportion of tracheids in the xylem and on the very variable thickening and lignification they show. Fibres are also differentiated in the phloem of all organs and, like the tracheids of the xylem, may become so thickened that their lumina are completely closed. Extremely thick-walled stone cells with branched pits in their walls are also formed in the phloem. Like the earlier formed phloem of the stem and root, the stem cortex becomes completely mechanical in function. It comes to consist entirely of thick-walled stone cells similar to those formed in the phloem. Many isolated stone cells are also formed in some of the cells of the pith of the stem and also in the cortex of the petiole (Pl. 3, figs. 5, 6; Pl. 4, figs. 9–11). As in the xylem, unevenness and paucity of lignification characterizes all these mechanical tissues. When treated with a lignin dye, a few cells may take a deep stain, but the majority stain either faintly or not at all (Pl. 4, fig. 1).

Stomata

In the leaf, stomata are confined to the lower surface, where they are exceptionally numerous (Pl. 5, fig. 2). In surface view, the guard cells are seen to be surrounded by two or more small variously shaped epidermal cells. In section, the guard cells are seen to be greatly thickened, leaving only a very small cavity in each. The cavity below the pore is also very small (Pl. 5, fig. 3).

Cork and lenticels

The origin of the cork has already been dealt with (cf. meristems). When formed it consists of radially seriated rows of cells with strongly suberized walls. As in most but not all members of the Myrtaceae, wide and narrow cells are produced alternately. In the clove, rings of cells almost square in cross-section alternate with rings of cells flattened in a radial direction, giving a stratified appearance (Pl. 5, figs. 4, 5).

In the cork of the stems are found many biconvex pores with a raised margin. The inner part of the pore is bounded by a meristematic layer continuous with the cork cambium. Immediately above this cambium are several layers of living cells

and above this in the centre of the lenticel are about twenty layers of radially seriated dead suberized cells. Very narrow air spaces run radially between the rows of dead cells. Towards the periphery of the lenticel the number of layers of dead cells is reduced as the edge of the lenticel is covered by the ruptured edges of the cork. At the surface of the pore is a more compact layer of cells which is level with the innermost layer of cork (Pl. 5, fig. 6).

Oil and oil glands

Globules of oil are found scattered in the parenchymatous tissues in all aerial parts of the tree (Pl. 3, fig. 8), and some cells in cortex and phloem may become blocked with it.

Solereder states that in a large number of genera of the Myrtaceae anatomical proof of the occurrence of secretory glands has been obtained, in others it has been inferred by the presence of pellucid dots in the leaves. In the clove, very numerous special glands are found in all aerial parts of the tree but not in the root. They occur equally towards both sides of the leaf where their formation begins soon after normal cell division has ceased. All do not develop quite simultaneously, and all stages in the development can be found in a single young leaf. Each gland arises by the division of a single epidermal cell to form a group of small cells which is pushed down between the parenchymatous cells below. Division ceases and the cells separate to form a cavity which at first has a distinct epithelium and a sheath formed from the flattened cells of the ground tissue. After some time the epithelium collapses and becomes suberized (Pl. 3, fig. 3). This collapse and suberization appear to be characteristic of the suborder, Myrtaceae (Solereder, 1908). In the cells of the sheath are often found oil globules. In the leaves, the glands persist and appear to remain functional throughout the life of the leaf, but in the cortex of the stem they sometimes become crushed as the surrounding tissues become sclerotic.

It has been reported that in the Myrtaceae the oil glands always have a pore to the exterior. In both sections and surface view, two cells which usually protrude slightly above the epidermal layer are seen to have a structure very similar to the guard cells of the stomata, but no section could be found where the pore was not covered with a layer of cuticle. From field observations also, it seems improbable that the leaves exude oil (Pl. 5, fig. 7).

Some non-living cell contents

No very exhaustive study was made of the non-living contents of the cells, but a few of the more obvious are listed below.

Tannin is secreted in large quantities in all parts of the tree and it is impossible to cut any part with a steel instrument without a blue colour developing. It fills cells in the cortex, phloem and xylem and often all the cells of the medullary rays are completely filled with it (Pl. 5, fig. 1). It is probably the oxidation of tannin to phlobophene that accounts for the red colour which rapidly develops in the wood

and bark of felled trees. Campbell seems not to have recognized tannin which is probably responsible for the blackening he describes in the phloem.

Saponin was found to be present in extracts made from the roots, but its exact location was not determined.

Calcium oxalate is also present in relatively large quantities, usually in the form of tetragonal crystals, although it occasionally occurs as raphides in the ground tissues of young roots. It is found in all tissues. Some cells of the mesophyll may be filled by groups of many small crystals. It also occurs in the pith, cortex and xylem but is most prevalent in the phloem, especially in the region of the node (Pl. 5, fig. 9).

Starch. Several workers have agreed that starch is never accumulated in substantial amounts in the healthy clove. It seems to occur in small quantities in the wood in all parts of the tree but its occurrence is variable. Campbell claims that it is absent from the roots of sudden death trees, but microscopical examination sometimes reveals it in the roots of suspect trees and, conversely, it may be absent from the roots of healthy trees. No attempt was made to study its distribution in detail nor to account for these inconsistencies.

Campbell states that the cells 'which contain starch in the healthy tree are found to be filled with a brown gummy substance in the "sudden-death" tree'. I have observed a brown granular substance in the wood and phloem of both healthy and suspect trees and it does appear sooner and in greater quantities in the latter. I have not identified it, but it might well be a degradation product of the starch.

Amorphous bodies. In sections of many leaves from seedlings, from healthy trees and suspect trees, are found bodies morphologically similar to the amorphous intracellular inclusions induced by viruses, such as strains of potato virus 'X' and of tobacco mosaic virus. They are large, usually nearly filling the cell, and they may occur in any of the parenchymatous tissues of the leaf but are usually most abundant in the cells immediately surrounding the vascular strand (Pl. 5, fig. 8). Whilst those induced by viruses usually consist mainly of protein, those formed by the clove contain little protein and, also in contrast to most of those due to virus infection, they are destroyed by fixatives containing alcohol. After a few minutes' immersion in water, part of the components dissolve away, leaving a hyaline vesicle containing a few granules. Owing to their instability, it was not possible to determine their exact constitution, but they were found to contain a little protein and some fat.

Possible causes of sudden death disease

Many suggestions, physical, physiological and pathological, have been made to account for this disease. Field observations led Nutman & Sheffield (1949) to discard all but a pathogenic hypothesis. As the immediate cause of death seems to be destruction of the absorbing roots, agencies which might cause this will be briefly considered.

Attack by such organisms as eelworms or insect larvae is eliminated by the rarity of those found in the clove plantations.

The symptom picture of sudden death and its pattern of spread are not incompatible with a bacterial pathogen. In all sections which have been examined, and most of which were prepared by techniques which would have shown bacteria had they been present, none were seen except in special circumstances.

Occasionally a root is found which for a few inches is swollen to about twice its normal diameter. When the swollen part is examined microscopically, a lesion full of bacteria is invariably found in the xylem. It happens that such roots have been found more often in healthy than in diseased areas. In any case, they are rare and seem to have no deleterious effect on the tree.

During the course of grafting experiments, W. B. May found that a large proportion of the seedlings raised in the Government nurseries were necrotic in the centre of the stem at the nodes. Further examination showed that the necrosis was not confined to the nodes, although most extensive there, but it also extended down the pith of the stem and patches of it occurred in the roots. In the stem the internal phloem, and in the root the external, might be affected. This necrotic tissue does not react with phloroglucinol. There is no evidence as to the ultimate fate of the affected seedlings, but this necrosis might well be a partial explanation of the high mortality rate of young seedlings in the plantations which has been reported by the Department of Agriculture. Bacteria were sometimes found in association with the necrosis, and it was thought that they might be the cause, but Rudd Jones (1949) has shown that this is not so. Indeed, he was unable to find any bacteria in the diseased seedlings he examined so, when present, they are presumably secondary invaders.

Campbell found bacteria in the roots of sudden death cloves, but, as the trees were already dead, it is probable that these also were secondary invaders.

That the etiological agent is a fungus was first suggested as long ago as 1907, and in 1922 Welsford claimed it was a species of *Peziza*. She produced no conclusive proof, and in 1940 Campbell thought the fungus she described was probably a *Melanconium* sp. and showed that it was very unlikely to be a parasite. Campbell isolated a large number of fungi from roots and also from the collar of wilted trees but was not able to obtain infection with any of them.

During the course of this work, fungal hyphae have been found in roots which have died back in both healthy and suspect trees (Pl. 5, figs. 10–12). In the individual root, no more were found in the suspect than in the healthy, and the total hyphae in the advanced suspect would seem to be greater only because a greater proportion of the roots die back.

It is difficult to evaluate Campbell's work, because he wrote only a report in very general terms and his scientific data are not available. He appears to have made isolations only from trees which had already wilted (i.e. they were already dead). Had any parasitic fungus been present, it might not have appeared in his cultures owing to the far greater prevalence, by that time, of the secondary saprophytic invaders. In order to bring this work into line with that of Campbell, wilted trees were examined. Material was collected from one of the Pemba outbreaks where the

spread is quite geometrical and adjacent rows are dying at regular 6-weekly intervals. Pieces of root, collar, trunk and stem from trees which had recently died were fixed. In the next row the trees were strongly suspect and similar material was taken from three of these and also from three, two or one tree in each of the next seven rows. Fungal hyphae, both hyaline and with brown contents, were found, side by side, in the vessels in all parts of recently dead trees. In the next, highly suspect row, the fungi were confined to the roots and were no more in quantity than those found in healthy roots. No attempt seems ever to have been made to isolate fungi from the roots of trees which were suspect but not yet dead. Especially when it is borne in mind that fungal hyphae are also found in the roots of presumably healthy trees, microscopical examination suggests that the fungi found in suspect trees would be quite insufficient to cause disease. Nevertheless, in the absence of proof of any other etiological agent, the matter would bear further investigation.

Comparisons have been made between sudden death of cloves and the Surinam disease of coffee. Stahel (1932) has claimed that this disease is caused by a flagellate which produces first hypertrophy and later necrosis of the phloem cells. The flagellate has not been grown in pure culture, but after osmic acid treatment large numbers of black fusiform bodies with apical flagellae, which were identified as a *Phytomonas* sp., were seen in the phloem 1 month before wilting and death of the infected tree. The present work confirms Campbell's in failing to find any abnormal inclusions in the phloem of cloves attacked by sudden death disease. Those described in coffee would bear further investigation. The photographs published do not differentiate them from other substances which might appear in the phloem cells as a result of the treatment received, and some of the detail shown in the drawings is considerably less than 0·2 μ in width and therefore could not have been observed in the optical microscope. Although the bodies are said to be present in both root and shoot, the disease has been transmitted by root but not by shoot grafts. In nature, the disease is thought to be transmitted by the Coccid, *Rhizoecus coffeae*, which feeds from the phloem of the root and lives in symbiosis with the ant, *Acropyga pickeli* (Bunzli, 1935). This mode of transmission has not, however, been proved, and Stahel considers *Lincus spathuliger* to be a more likely vector. The two diseases do offer many interesting parallels; the variation from acute to chronic forms; the necrotic phloem followed by destruction of the absorbing roots, death by sudden wilting, and the possible interrelationships between the host, the disease, the coccid and its attendant ant (Nutman & Sheffield, 1949). But for the descriptions of the phloem flagellate, the Surinam disease of coffee would be interpreted as due to a virus confined to the roots.

In its progress, the sudden death disease shows many similarities to proved virus diseases such as quick decline of citrus, phloem necrosis of elm, etc. For a number of reasons (Nutman & Sheffield, 1949) it seems most likely that the pathogen causing the disease is a virus, but microscopic examination has done little either to support or refute this hypothesis. With its small amount of functional vascular tissue, its

enormously developed mechanical system, its almost total lack of air-spaces even in the assimilating tissues and the large deposits of waste materials, the clove is far from being a typical dicotyledon. A few of the abnormalities shown by the apparently healthy clove are characteristic of the genus, but others are those more usually associated with a diseased condition, either pathogenic or physiological. As most of these are accentuated when a tree becomes suspected of sudden death and as it cannot at present be definitely proved that the controls were, in fact, healthy, it might be argued that the disease is due to an extremely slow-acting pathogen. There is now some evidence that the period elapsing between infection and death of mature trees in some outbreaks exceeds 12 months, but the simultaneous death of all trees in a row in some outbreaks showing peripheral spread (Nutman & Sheffield, 1949) suggests that the period cannot be numbered in years. The district from which presumed healthy material was collected was inspected over a year later and no suspect trees were found within several miles. Seedlings from Government nurseries also invariably showed the abnormalities described. It seems very improbable, therefore, that the abnormalities found in healthy material can be due to an incipient infection. As little is known of the nutritional requirements of the clove, it is possible that they are due to unsuitable or exhausted soils.

If disturbance of the plastids be excepted, it is probably true to say that it is the minority of plant viruses which cause visible abnormalities in the cells. Those histological abnormalities usually associated with virus infection have been fully discussed by Esau (1938, 1948*a*) and need be but briefly considered here.

The most characteristic are the intracellular inclusion bodies, all of which contain protein and some of which are known to contain virus (Sheffield, 1939, 1941, 1948). In both healthy and diseased cloves, bodies morphologically similar to some of these have been found, but chemically they are different and might be analogous to the bodies which have been experimentally produced (Sheffield, 1934) by treatment with molybdates and shown to be compounds of the mineral with tannins or anthocyanin (Warington, 1937). However, these bodies contained far less tannin than those experimentally produced, although clove tissues are rich in tannins.

Intranuclear inclusions are said to arise from a variety of causes but are rare as a symptom of plant virus infection having only twice been reported (Kassanis, 1939; McWhorter, 1941). No abnormalities were found in the nuclei, at any stage, in either healthy or suspect cloves.

The necrosis and gummy deposits found in many tissues of the clove are similar to those produced either by virus infection or by mineral deficiencies. Although obliteration is common, necrosis of the phloem is most often the result of virus infection, as in leaf-roll of potato, where it is diagnostic (Sheffield, 1943). Bond (1947) has shown that phloem necrosis does occur in healthy tea plants. Tea may also become infected with a virus which causes phloem necrosis. Bond finds that, in most tissues, this necrotic phloem cannot be distinguished from that found in healthy plants. In only the petiole, the two forms, true and false necrosis, are

differently distributed and can therefore be used in diagnosis. In the clove, this distinction cannot be made, for, although the amount of necrotic phloem seems to be increased as a result of the disease, the distribution seems to be random in all organs in both healthy and suspect plants. Phloem necrosis is also associated with boron deficiency, but spectrographic analysis has shown that this is not likely to be the cause in the clove (Wallace, 1945).

Occlusion of the xylem vessels by brown, granular and gummy deposits and by tyloses is more prevalent in suspect than in healthy cloves. This might be caused by any disturbance of the translocatory system, but Esau (1948*b*) has found a similar state of affairs associated with known virus diseases. She found that Pierce's disease virus induces excessive and precocious development of tyloses in the xylem of the vine, and both this virus and that of phoney peach disease cause the production of gummy deposits in the xylem.

Hutchins (1933) found that, if sections of peach roots are soaked in acidulated methyl alcohol, blue patches appear in the xylem of those infected with phoney peach virus but not in the healthy. Unfortunately, the chemistry of this reaction is not understood. The test was tried on clove roots of $\frac{1}{4}$–$\frac{3}{4}$ in. diameter but no reaction was obtained from either healthy or suspect trees.

No histological feature was found which would serve as a diagnostic character of sudden death disease of cloves and so enable it to be recognized at an earlier stage than is at present possible. All the abnormalities seen in the suspect are present to a less extent or appear later in the apparently healthy tree. If considered alone, the evidence presented here might suggest that sudden death is merely the culmination of a long period of unsuitable nutrition or conditions of growth. However, the evidence of field observations suggests very strongly that the disease is due to a pathogen (Nutman & Sheffield, 1949) and nothing in this paper is contrary to that hypothesis.

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The unpublished reports referred to are in the files of the Department of Agriculture, Zanzibar. My thanks are due to the Director for allowing me access to them and to quote from them.

EXPLANATION OF PLATES 3–5

All photomicrographs were taken with a Leitz 'Makam' camera, the source of illumination being a Pointolite screened by a Wratten filter. A Leitz periplanatic ocular (× 10) was used throughout, and the objectives used were a Hawkesley 1 in., a Leitz 3*b*, a Leitz 6L and a Leitz 2 mm. apochromatic (N.A. 1·4) giving magnifications of × 75, × 140, × 650 and × 900 on the negatives. The condenser was a Zeiss aplanatic (N.A. 1·4) but, except when an oil immersion objective was used, the top lens was removed.

All figures were photographically enlarged to × 1½ and have been reduced by one-half in reproduction.

After the description of each figure, there is given in brackets: the final magnification, the stain used in preparing the section and the number of the Wratten filter used in taking the photograph; in that order.

Plate 3

Fig. 1. Longitudinal section through the growing point of a healthy stem showing the primordial and primary meristems. Young leaves are wrapped around the stem apex and, in these, cell division has ceased at the base but still continues at the top. In the axils of the second pair of leaves are one and two buds, one of which is dead. (× 55 Heidenhain's haematoxylin; B and ground glass.)

Fig. 2. Longitudinal section of the growing point of a root from a healthy tree, showing the

primordial meristem, the differentiation of the primary meristem and the layer giving rise to the root cap. (×485; iodine crystal violet and fast green; B.)

Fig. 3. Transverse section of healthy leaf less than 1 in. long. The ground tissue is not yet differentiated. Three stages in the development of oil glands are shown: *a*, the initial has divided into a number of closely packed cells with very dense contents; *b*, the cavity is beginning to form; *c*, the epithelium has collapsed and disintegrated. A sheathing layer can be seen in all three stages. (×485; safranin and Delafield's haematoxylin; B.)

Fig. 4. Transverse section of part of midrib of healthy leaf less than 1 in. long. Each vascular bundle consists of a group of two to three vessels with phloem cells above and below. (×485; iodine crystals violet and erythrosin; B.)

Fig. 5. Transverse section of a green leaf from a healthy tree passing through a small vein. It shows a small-celled epidermis with a thick cuticle, elongated palisade cells containing chloroplasts and mesophyll cells with very small air-spaces. Some of the palisade cells are dead. One of the mesophyll cells contains crystals of calcium oxalate. The vascular bundle consists of xylem vessels, some of which are blocked; with phloem above and below. The outer phloem is converted into fibres so that a ring of sclerotic tissue completely surrounds the bundle. Some of the thin-walled phloem cells are blocked and others are necrotic. (×485; iodine crystals violet and erythrosin; B.)

Fig. 6. Transverse section of half of the midrib of a young yellow leaf from a suspect tree. There is a continuous group of xylem vessels across the centre and this is completely surrounded with phloem, the outer cells of which are sclerotic. A few of the vessels are becoming blocked and there is obliteration and necrosis in the phloem. (×485; iodine crystal violet and erythrosin; B.)

Fig. 7. Transverse section of part of the midrib of an old healthy leaf showing considerable necrosis in the phloem. (×485; iodine crystal violet and fast green; A.)

Fig. 8. Transverse section of an old leaf from a suspect tree. The plastids have disintegrated. Oil globules are present in many of the cells and amorphous inclusion bodies in three mesophyll cells near the centre of the photograph. (×485; iodine crystal violet and erythrosin; H.)

Fig. 9. Transverse section of a young stem from a suspect tree. The section passes through the cambium which consists of three layers of very thin-walled cells. Xylem is to the top and phloem to the bottom of the photograph. (×485; safranin and fast green; B and E.)

Fig. 10. Longitudinal section of the stem of a healthy tree, passing through the cambium. Xylem is to the top and phloem to the bottom of the photograph. (×485; safranin and fast green; B.)

PLATE 4

Fig. 1. Transverse section of the stem of a healthy tree showing uneven thickening and lignification of the xylem. (×485; safranin and fast green; C.)

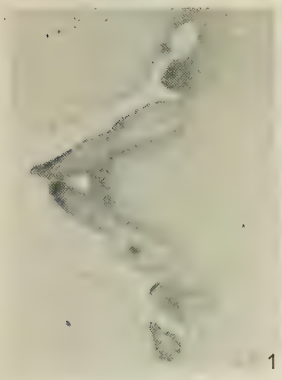
Fig. 2. Transverse section of a healthy root to show tyloses in the xylem. (×485; safranin and fast green; E.)

Fig. 3. Longitudinal section of root of a suspect tree showing xylem element completely blocked with tyloses. (×485; safranin and fast green; E.)

Fig. 4. Transverse section of the midrib of a flecked leaf from a suspect tree. The xylem vessels are already mostly blocked. Some of the phloem cells are blocked, others are obliterated or necrotic. (×485; iodine crystal violet and erythrosin; B.)

Fig. 5. Transverse section through the internal phloem of a young stem. Secondary thickening has commenced and some fibres are differentiated. (×485; safranin and fast green; B.)

Fig. 6. Transverse section through the phloem of the leaf of a suspect tree showing blocking with oil, obliteration and necrosis. (×675; iodine crystal violet and erythrosin; B.)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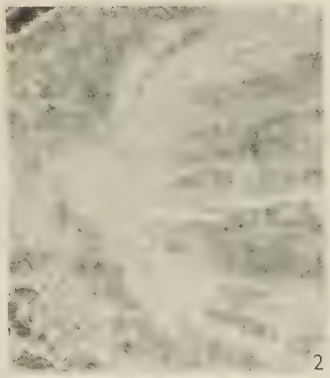
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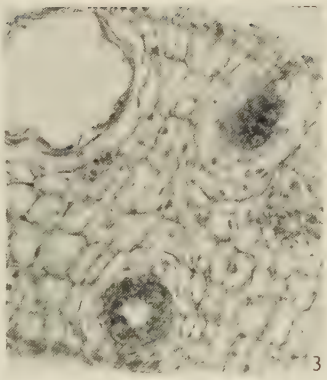
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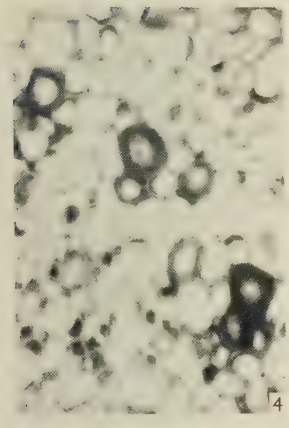
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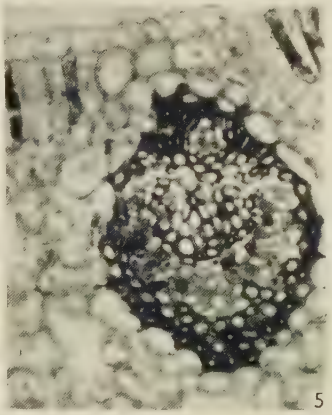
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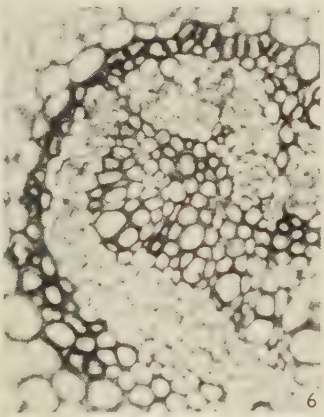
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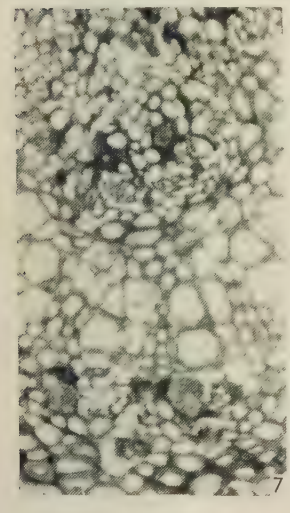
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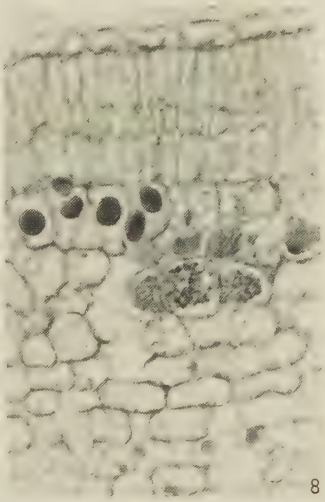
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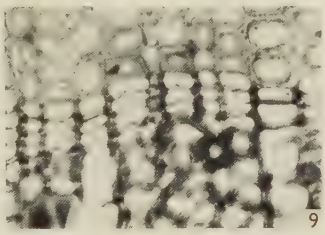
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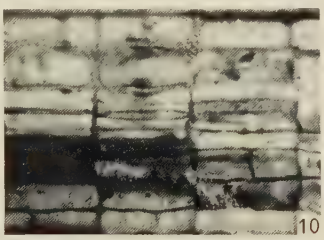
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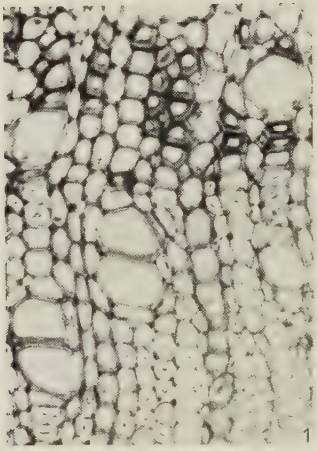
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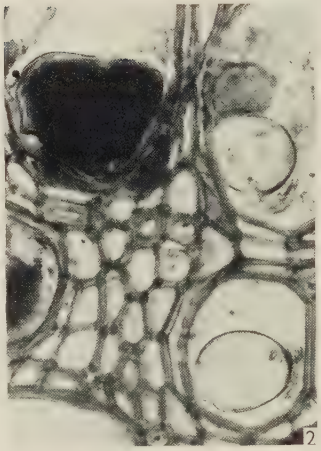
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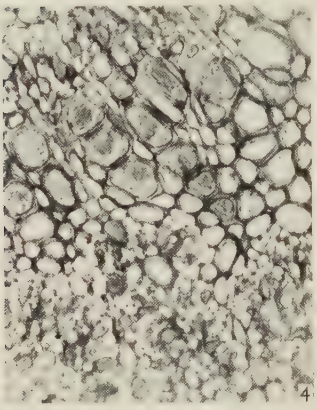
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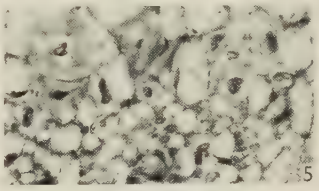
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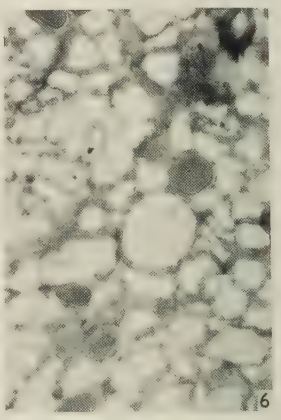
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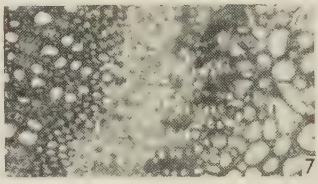
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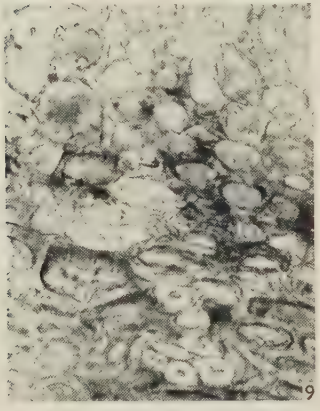
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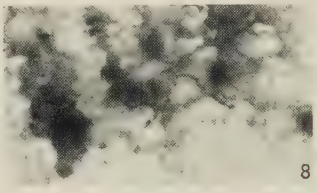
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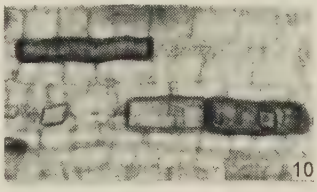
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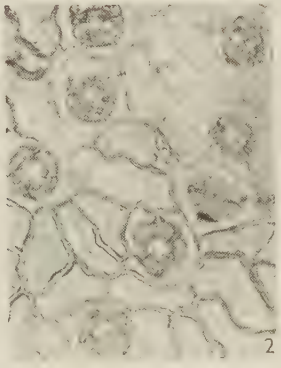
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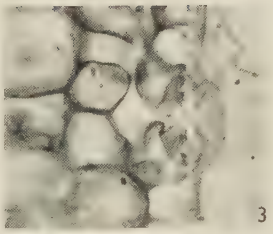
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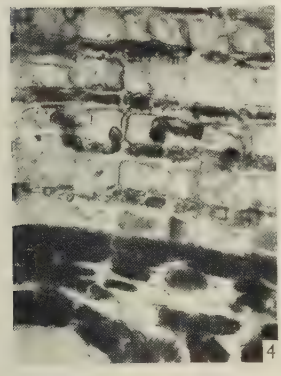
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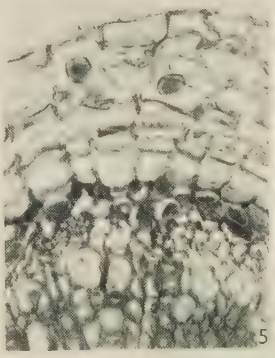
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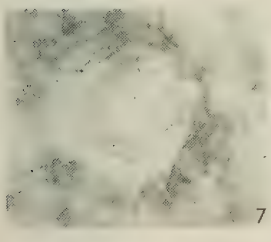
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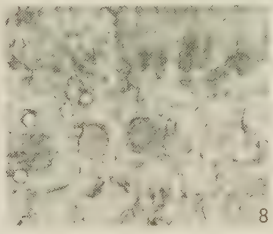
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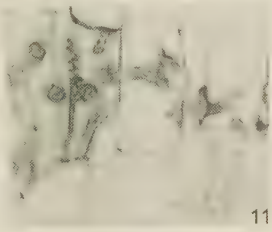
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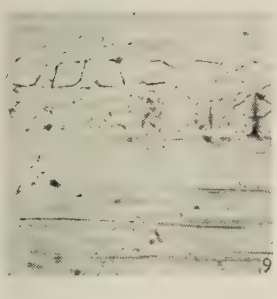
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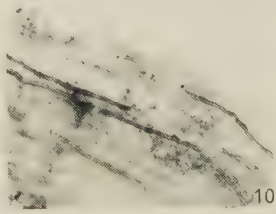
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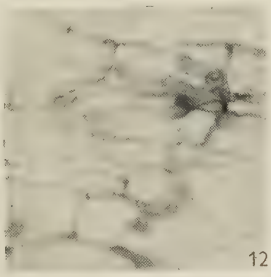
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Fig. 7. Transverse section of a young stem passing through the internal phloem. This stem is slightly older than that shown in fig. 5 and the phloem nearest the pith is mainly necrotic. (The pith is to the right of the photograph.) (× 105; phloroglucinol; B and ground glass.)

Fig. 8. Part of the field of fig. 7 to show the necrotic phloem. (Pith is to the top of the photograph.) (× 485; phloroglucinol; B.)

Fig. 9. Transverse section of stem to show formation of mechanical tissue in the outer phloem and cortex. (× 485; phloroglucinol; B.)

Fig. 10. Longitudinal section of pith of stem showing small rectangular parenchymatous cells and lignified stone cells. A few crystals of calcium oxalate can be seen. (× 105; phloroglucinol; B and ground glass.)

Fig. 11. Longitudinal section of root through outer part of phloem to show formation of stone cells and fibres. (× 485; phloroglucinol; B.)

PLATE 5

Fig. 1. Longitudinal section through the collar of a suspect tree, showing a medullary ray passing across the xylem. The cells are filled with tannin which shows up the protoplasmic connexions between the cells. (× 485; safranin and fast green; B.)

Fig. 2. Surface section of the lower epidermis of a green leaf showing the very numerous stomata and small epidermal cells. (× 485; living, unstained; B.)

Fig. 3. Transverse section of lower epidermis of a green leaf showing a stoma and very small cavity below. (× 675; iodine crystal violet and erythrosin; B.)

Fig. 4. Transverse section of young stem through outer cortex and cork. The layers of cork are striated, rectangular cells alternating with cells very narrow in a radial direction. (The space in the left bottom corner of the photograph is part of an oil gland.) (× 485; safranin and fast green; E.)

Fig. 5. Transverse section of young root showing the endodermis, the beginning of cork formation and the drying up of the cortex and epidermis. (× 485; safranin and fast green.)

Fig. 6. Longitudinal section of stem passing through a lenticel. (× 55; safranin and fast green; B and ground glass.)

Fig. 7. Oil gland as seen in surface section. It projects above the epidermis and, at this level, consists of two cells with dense contents. (× 485; living, unstained; B.)

Fig. 8. Transverse section of healthy leaf showing amorphous inclusion bodies in mesophyll cells. (× 485; living, unstained; B.)

Fig. 9. Crystals of calcium oxalate in a longitudinal section of phloem of healthy stem. (× 485; safranin and fast green; B and E.)

Fig. 10. Fungi in the ground tissue of an absorbing root from a suspect tree. (× 485; iodine crystal violet and erythrosin; B.)

Fig. 11. Fungi in ground tissue of absorbing root from a healthy tree. (× 485; iodine crystal violet and erythrosin; B.)

Fig. 12. As fig. 11. Some of the fungi appeared blue; others, red in this preparation. (× 485; iodine crystal violet and erythrosin; B.)

(*Received* 18 *June* 1949)

SOME EFFECTS OF HOST NUTRITION ON THE SUSCEPTIBILITY OF PLANTS TO INFECTION BY CERTAIN VIRUSES

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(With Plate 6)

Fertilizer treatments that greatly influenced the growth of tobacco and potato plants in pots had little effect on the number that became infected with potato virus Y when the plants were colonized by equal numbers of infective aphids, though the number was slightly decreased by nitrogen and increased by phosphorus.

The number of local lesions produced on leaves of tobacco and *Nicotiana glutinosa* by tomato aucuba mosaic and tobacco mosaic viruses was increased by additions of both nitrogen and phosphorus, provided that these also increased growth. The predominant effect of both nutrients in increasing susceptibility was indirect by increasing plant size, but over certain critical ranges both elements also increased the numbers of lesions produced per unit leaf area. Conditions of maximum susceptibility approximated closely to those producing optimal growth, and susceptibility, whether measured by lesions per half-leaf or per unit area, was decreased by a deficiency or excess of either element. Sometimes the addition of nitrogen reduced susceptibility when still increasing plant growth.

Although the literature contains many statements about the effects of host nutrition on the susceptibility of plants to viruses, the subject has received little detailed or critical study. Most writers have regarded sensitivity as synonymous with susceptibility, and their statements deal solely with effects on the type and severity of symptoms evinced by infected plants. Sensitivity, however, indicates only the extent to which individual plants are affected, and although records of host reactions may provide an attractively simple method of assessing susceptibility, the results are limited and may be misleading. Sensitivity is only one of the qualities of host plants included in the general term susceptibility, and its relation with others, such as liability to become infected or suitability to maintain a high virus concentration, is largely unknown. There is, however, no reason to assume that a change which better enables a plant to tolerate the presence of a virus will also render it less likely to become infected or less favourable a medium for virus multiplication. Before the effects of host nutrition on susceptibility can be interpreted at all adequately, a knowledge is needed of effects on all host qualities that might influence the rate at which viruses spread. The experiments described in this paper were made to determine in what variations in the amounts of nitrogen, phosphorus and potassium supplied to plants affected their susceptibility to infection by certain viruses; effects of such variations on the multiplication of the viruses in infected plants will be described separately.

The only previous quantitative work on this problem seems to be that done by Spencer (1935, 1935*a*) with strains of tobacco mosaic virus. His experiments were made mostly with tobacco (*Nicotiana tabacum* var. Turkish) grown in infertile soil to which nutrient solutions were added at regular intervals, the amounts of nitrogen, phosphorus or potassium in the solutions being varied while other constituents remained constant. Variations in the amounts of nitrogen supplied to *N. glutinosa* grown in soil and to French bean (*Phaseolus vulgaris* var. Golden Cluster) grown in sand were also studied (1935). Effects on susceptibility were measured by counts of local lesions; beans were infected by rubbing leaves over their whole surface with infective sap diluted 1/100 and tobacco and *Nicotiana glutinosa* by pricking leaves with mounted sets of twenty needles dipped in undiluted infective sap. The effects of varying nutrition on plant growth were estimated by weighing sample lots of plants. From comparisons of the weights with lesion counts, Spencer concluded that growth rate and susceptibility to infection were not necessarily correlated, and that different nutrients affected the two phenomena differentially. He concluded that nitrogen specifically increased susceptibility even when applied in amounts above that which produced maximum growth, whereas phosphorus increased susceptibility only while it was also increasing growth and potassium decreased susceptibility at amounts that were still increasing growth. The needle-method used for inoculation in most experiments produced few lesions and the differences in lesion counts on which these conclusions were based were often small. No statistical analysis of the results was given, so that the significance of the differences is uncertain, and the chief unequivocal result seems to be that an acute deficiency or a gross surplus of any of the elements reduced both plant growth and susceptibility to infection.

Materials and methods

The viruses and host plants used were potato virus Y in tobacco (*N. tabacum* var. White Burley) and potato (*Solanum tuberosum* var. Majestic), tomato aucuba mosaic virus in tobacco and tobacco mosaic virus in *Nicotiana glutinosa*. Infections with virus Y were made using *Myzus persicae* Sulz.; the aphids were starved for 4 hr. before use, fed for 2–3 min. on detached tobacco leaves infected with virus Y, and then transferred immediately to the test plants, which were fumigated with nicotine vapour 12 hr. later. Infections with the two strains of tobacco mosaic virus were made by rubbing the upper surfaces of the leaves as uniformly as possible with the forefinger wet with inoculum. Infective sap diluted with water was used as inoculum; to obtain numbers of lesions in the range in which differences are most nearly proportional to virus concentration, two dilutions of inoculum were used, one being rubbed over the left-hand halves of the leaves and the other over the right-hand halves.

No standard description can be given of the manner in which the test plants were grown and the nutrients added, for conditions varied considerably in different experiments. Seed was sown in potting compost and seedlings were transplanted

at the four-leaf stage to unglazed pots containing 1 kg. of potting medium, and the differential treatments were applied from this time. In some experiments the medium was pure sand, in others mixtures of equal quantities of sand and infertile soil and, most often, mixtures containing 50% infertile soil and 25% respectively of sand and peat. The nitrogen, phosphorus and potassium contents of the last mixture were respectively 0·15, 0·001 and 0·01%. When sand was used, the nutrients were always added in solution; the nutrient solutions were the same as those described by Spencer (1937) and 100 ml. were given twice weekly. In some experiments with soil, nutrients were also applied as solutions, but with most of these the fertilizers were added in solid form and mixed thoroughly with the potting medium before the seedlings were transplanted. The amount added for each plant receiving a supplement of nitrogen was 2·82 g. $(NH_4)_2SO_4$, of phosphorus 0·76 g. $Ca(H_2PO_4)_2$, $2H_2O$ and of potassium 1·12 g. K_2SO_4. The pots were stood in saucers to prevent loss of nutrients by leaching and the various treatments were arranged at random over the glass-house benches to minimize differences arising from positional effects. The different fertilizers produced plants of greatly differing sizes, so that uniform watering was impossible and plants were watered according to normal horticultural practice. Experiments were made at various times of the year; in winter the houses were kept at about 18° C. and the plants also received extra illumination; in summer the temperature often exceeded this, but plants were rarely exposed to direct sunlight for long periods as the houses were shaded when this was thought desirable.

In experiments with plants grown in sand, the amounts of either phosphorus or nitrogen supplied were varied while all other nutrients were kept constant. In experiments with plants grown in mixtures containing soil, a factorial design was used to provide information on the effects of changing the level of each nutrient on the response to the others. There were usually eight treatments: no fertilizer added (1), nitrogen alone (*n*), phosphorus alone (*p*), potassium alone (*k*), nitrogen and phosphorus (*np*), nitrogen and potassium (*nk*), phosphorus and potassium (*pk*), and nitrogen, phosphorus and potassium (*npk*). The data were analysed statistically by the method described by Yates (1937), which gives the results in the form of main affects (N, P and K) and their interactions (N.P, N.K, P.K and N.P.K). The main effect of a treatment is one-quarter of the difference between the sum of the values of the four sets receiving that treatment and the sums of the values of the four not receiving it, while the interaction between two factors is the difference in response to one in the presence and absence of the other, e.g. the main effect of nitrogen $(N) = \frac{1}{4}(n+np+nk+npk) - \frac{1}{4}(1+p+k+pk)$ and the interaction between nitrogen and phosphorus $(N.P) = \frac{1}{4}(1+k+np+npk) - \frac{1}{4}(n+nk+p+pk)$, where *n*, *np*, etc., are the mean values for the replicates receiving the different treatments.

The customary method of recording local lesions as numbers per leaf is clearly inadequate when treatments applied have large effects on leaf size. Lesions were therefore recorded in two ways; the number per half-leaf was counted, the area of the leaf was then estimated and the number of lesions per sq.cm. was calculated.

Leaf area was calculated from the method described by Sadasivan (1940), the length of the leaf being multiplied by its width at the widest point and the product multiplied by 0·7 for tobacco and 0·88 for *Nicotiana glutinosa*. Leaf area was also often employed as an index of plant growth, though sometimes the fresh weight of plants was also determined; the two measurements agreed closely. Numbers of local lesions are not normally distributed and so are not directly suitable for applying statistical tests of significance (Kleczkowski, 1949); before analysis, therefore, all counts were increased by one and transformed into logarithms.

Results

Effects on plant growth

Strikingly different responses to the added nutrients were obtained with plants grown in soil mixtures and those grown in sand. With the former, phosphorus had by far the greatest effect in increasing growth, whereas in the quantities applied to sand it was largely ineffective and nitrogen produced much greater effects. In experiments made at different times with the same potting medium, responses duplicated each other fairly closely, though there were some seasonal effects; with plants grown in soil mixtures, for example, phosphorus appeared to stimulate growth more in winter than in summer, whereas the reverse was true with nitrogen.

The quantitative responses to the different treatments are indicated by the measurements of wet weights and leaf areas recorded in the various tables. Here we need consider only effects on the appearance of plants. With tobacco and *N. glutinosa* grown in the soil mixtures, potassium had no obvious effects, except in the presence of nitrogen and phosphorus, and the *npk* treatment usually produced plants of a better and more uniform colour than any other. With potato, however, potassium was highly beneficial, increasing growth and preventing marginal leaf scorch. The effects of phosphorus and nitrogen on size and colour of leaves of tobacco plants grown in the soil-sand-peat mixture, and on the type of lesion produced by tomato aucuba mosaic virus, are illustrated in Pl. 6. The leaves are of similar ages and from comparable positions from plants receiving *k*, *nk*, *pk* and *npk* treatments; the four treatments without potassium were essentially similar. With plants grown in these conditions, phosphorus increased rate of growth and leaf size, and also stimulated the formation of flowering stalks; nitrogen mainly influenced leaf colour, and in the absence of phosphorus usually reduced growth and leaf size, but in its presence increased both. The individual plants within each treatment resembled one another closely, and differences produced by the various treatments can be summarized as: treatments (1) and *k* gave slow-growing plants, which were rather pale but showed no obvious abnormalities; *n* and *nk* gave smaller plants than (1) and *k*, with dark green leaves, the lowest being sometimes tinged with orange; *p* and *pk* gave rapidly growing, tall plants, with pale leaves, the lowest often being wholly chlorotic; *np* gave larger plants than *p* and *pk*, with dark green leaves, though sometimes the lowest showed slight signs of potash deficiency, having a bluish tinge and

shortened mid-ribs; *npk* usually produced the best-looking plants, similar in size to *np*, but free from any discolorations or deformities.

The effects of these various treatments on the composition of tobacco leaves have been described by Holden & Tracey (1948). Nitrogen and phosphorus increased wet weight of the plants, total dry matter and total nitrogen and phosphorus contents, but in other respects the two nutrients were opposed. Phosphorus increased the carbohydrate components and protease activity, whereas nitrogen increased protein and pectase.

Susceptibility to infection with potato virus Y

Preliminary experiments to determine favourable conditions for growing and fertilizing test plants were used to determine effects of nutritional differences on susceptibility to infection by virus Y introduced by aphids. At first a sand-soil potting medium was used, and three levels of nitrogen, phosphorus and potassium were applied in all combinations. There were five replications of each of the twenty-seven different treatments, and nutrients were added in solution twice weekly at the rates, in mg. per day, of nitrogen 0, 5 and 25, phosphorus 0, 1·3 and 6·5, and potassium 0, 5 and 25. Three experiments of this type were made with tobacco and four with potato plants. Infections were made at periods of between 8 and 10 weeks after the treatments were begun, two aphids per plant being used with tobacco and five with potato. The pooled results from the various experiments are summarized in Table 1, which shows the total numbers of infected plants and the mean wet

Table 1. *Mean effects of different levels of nitrogen, phosphorus and potassium on growth of tobacco and potato plants and on infection with virus Y*

	Tobacco		Potato	
Treatment*	Total plants infected out of 135 colonized	Mean wet weight of plants (g.)	Total plants infected out of 180 colonized	Mean wet weight of plants (g.)
No nitrogen	84	17·2	77	34·0
Medium nitrogen	88	33·5	75	47·4
High nitrogen	92	44·1	71	27·4
No phosphorus	87	15·9	80	25·3
Medium phosphorus	83	29·9	60	38·1
High phosphorus	94	48·9	83	45·2
No potassium	92	26·6	79	25·0
Medium potassium	82	33·5	75	38·6
High potassium	90	34·7	69	45·0

* Pooled results for all three levels of other nutrients.

weights of plants obtained at each level of the three elements. Despite considerable effects on the growth, there are no apparent effects on the numbers of infections obtained.

These preliminary tests revealed limitations and disadvantages of the techniques. Adding the fertilizers repeatedly in solutions produced a crust on the soil surface

and the mixture of sand and soil used lost water rapidly; also, with twenty-seven different treatments, the number of infected plants with each treatment was too small for statistical analysis of the results to be satisfactory. The method was therefore altered to growing plants in the mixture of soil, sand and peat and the fertilizers were added in solid form to the potting medium at the time of mixing. The number of treatments was reduced to eight, all combinations of the presence and absence of nitrogen, phosphorus and potassium, and the number of replications of each treatment was increased to ten. The results from four experiments of this type with tobacco plants are given in Table 2. Infections were made by placing one aphid on

TABLE 2. *Effect of fertilizers on growth of tobacco plants and infection with potato virus Y*

Treatment	Mean weights of plants (g.)					No. of plants infected out of ten				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Mean	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Mean
(1)	18	37	49	42	36	2	6	6	8	5·5
n	14	41	56	47	39	1	6	5	9	5·3
p	46	53	85	71	64	4	7	9	10	7·5
k	21	39	49	49	39	2	8	9	7	6·5
np	72	98	127	120	104	1	6	5	7	4·8
nk	14	48	54	64	45	2	6	6	7	5·2
pk	52	62	92	77	71	1	5	7	6	5·8
npk	99	125	154	165	136	3	5	8	7	5·8

each test plant, and it is again obvious that any effects of nutrition on the numbers of plants becoming infected are small relative to their effects on plant growth. Statistical analysis on the wet weights of the plants and (after transformation into angular degrees) on the percentages of plants that became infected merely confirmed this conclusion. With plant weights all the main effects and interactions were positive and significant at the 1% probability level, the main effects of P, N and K being respectively forty, twenty and eight times the standard error, whereas with infections the main effect of N was negative and significant at the 5%, and the main effect of P was positive and just not significant at the 5% level.

These results suggest that the intrinsic susceptibility of potato and tobacco leaves to infection with potato virus Y introduced by aphids is not greatly altered by differences in nutrition that have large effects on plant growth. The relevance of these results obtained under glass, from plants colonized with equal numbers of aphids, to conditions in the field is uncertain. As van der Plank (1947) has pointed out, size of plant is itself a factor that may well influence the rate at which viruses spread. A large plant has greater chance than a small one of contracting infective aphids, and, once infected, it will supply a larger total volume of infected plant tissues from which spread to other plants can occur. Size of plant and host nutrition may also influence spread by affecting the size of aphid infestations or the frequency with which aphids move from one plant to another. In field experiments at Rothamsted with potatoes receiving different fertilizer treatments, there has always been a greater

spread of virus Y in plots receiving supplements that increase the size of haulm than in plots where some nutrient was deficient and limiting growth. This can probably be attributed to one or other of these indirect effects rather than to a change in the physiology of individual host cells that render the establishment of the virus more likely to occur.

Tobacco mosaic viruses on tobacco and Nicotiana glutinosa

Table 3 shows the results of three experiments with tomato aucuba mosaic virus, inoculated to tobacco plants grown in soil-sand-peat mixtures to which the fertilizer supplements were added before the plants were potted. The dates at which plants were potted and inoculated in the three experiments were: Exp. 1, 30 November and 5 March; Exp. 2, 20 March and 6 May; and Exp. 3, 1 August and 24 September. There were eight replicates of each treatment in Exp. 1, ten in Exp. 2, and five in Exp. 3; in each experiment, three leaves of comparable ages and positions were inoculated on every plant, the left-hand halves being rubbed with infective tomato sap diluted 1/100 and the right-hand halves with sap diluted 1/500. The different fertilizer treatments produced similar effects at both dilutions, and only the results

Table 3. *Effects of fertilizer treatments on leaf area of tobacco plants and lesions produced by aucuba mosaic virus*

	Exp. 1			Exp. 2			Exp. 3		
Main effects and interactions	Leaf area (sq.cm.)	Average lesions per half-leaf†	Average lesions per sq.cm.‡	Leaf area (sq.cm.)	Average lesions per half-leaf	Average lesions per sq.cm.	Leaf area (sq.cm.)	Average lesions per half-leaf	Average lesions per sq.cm.
Mean	128·2	1·78	−0·24	289·0	1·70	−0·74	201·8	1·59	−0·59
N	12·6	−0·08	−0·04	69·6**	0·02	−0·07	5·3	−0·31**	−0·33**
P	130·3**	0·89**	0·37**	124·1**	0·62**	0·44**	22·9	0·67**	0·07
K	−7·5	0·10	0·14*	47·8**	0·15*	0·08	3·8	0·16	0·07
N.P	37·0**	0·26**	0·11*	73·4**	0·04	−0·06	7·2	0·29**	0·14
N.K	−19·8**	−0·16*	−0·11*	10·4	0·05	0·05	1·9	0·20*	0·13
P.K	5·2	−0·12	−0·17*	23·6*	0·06	0·03	2·6	−0·12	−0·12
N.P.K	−20·4**	−0·08	−0·03	−3·4	−0·15	−0·13	0·2	−0·08	−0·03
Standard error	6·41	0·066	0·050	9·02	0·065	0·066	16·6	0·086	0·099
Degrees of freedom	56	56	56	72	72	72	32	32	32

* Significant at the 5 % level. ** Significant at the 1 % level.

† Lesions (x) expressed as $\log_{10} x+1$. ‡ Lesions (x) expressed as $\log_{10} \frac{x+1}{\text{leaf area}}$.

with sap at 1/100 are recorded. The table shows the main effects and interactions on leaf area and on numbers of local lesions (increased by one and transformed into logarithms) per half-leaf and per sq.cm. The effects on leaf area varied in different experiments, presumably because of seasonal differences in the ability of the plants to utilize the different nutrients, but phosphorus always increased it most. Phos-

phorus, too, consistently increased the numbers of lesions, both per half-leaf and per unit area, though the increase per unit area in Exp. 3 was not statistically significant. Nitrogen consistently reduced the numbers of lesions per unit area, though the reduction was usually not statistically significant. There was a positive interaction between nitrogen and phosphorus on leaf area and numbers of lesions per half-leaf, but little effect on numbers per unit area. The main effect of potassium, and its interactions, were relatively small and variable.

TABLE 4. *Effects of fertilizers on leaf area of* Nicotiana glutinosa *and numbers of lesions produced by tobacco mosaic virus*

	Exp. 1			Exp. 2			Exp. 3		
Main effects and interactions	Leaf area (sq.cm.)	Average lesions per half-leaf†	Average lesions per sq.cm.‡	Leaf area (sq.cm.)	Average lesions per half-leaf	Average lesions per sq.cm.	Leaf area (sq.cm.)	Average lesions per half-leaf	Average lesions per sq.cm.
Mean	141·3	2·21	0·14	160·5	1·79	−0·31	78·2	1·44	−0·38
N	23·2*	−0·36**	−0·37**	−1·2	−0·42**	−0·38**	−13·7	−0·42**	−0·32**
P	146·6**	0·20**	−0·30**	148·0**	0·58**	0·06	72·7**	0·67**	0·20*
K	5·1	−0·02	−0·03	4·8	0·09	0·11	6·7	0·00	−0·01
N.P	36·1**	0·10	−0·01	56·1**	0·46**	0·30**	−1·7	0·52**	0·48**
N.K	3·1	0·01	0·02	−7·5	0·06	−0·03	1·6	0·23*	0·20*
P.K	4·7	0·04	0·04	5·9	0·07	0·02	12·2	0·13	0·05
N.P.K	8·2	0·05	0·03	−24·6*	−0·10	0·03	−1·2	−0·22*	−0·20*
Standard error	9·60	0·057	0·050	9·96	0·10	0·082	6·93	0·097	0·079
Degrees of freedom	24	24	24	32	32	32	24	24	24

* Significant at the 5 % level. ** Significant at the 1 % level.

† Lesions (x) expressed as $\log_{10} x+1$. ‡ Lesions (x) expressed as $\log_{10} \frac{x+1}{\text{leaf area}}$.

Table 4 shows the results from three similar experiments with *N. glutinosa* grown in soil-sand-peat mixtures and inoculated with tobacco mosaic virus. Dates for potting and inoculating plants in the three experiments were: (1) 1 March and 6 May, (2) 15 May and 10 July, and (3) 6 September and 6 November. In Exp. 1, there were four plants per treatment and five leaves per plant were inoculated; in Exp. 2, there were five plants per treatment and six leaves per plant were inoculated; in Exp. 3, there were four plants per treatment and five leaves per plant were inoculated. The results closely parallel those obtained with tobacco plants and tomato aucuba mosaic virus. Phosphorus consistently increased the leaf area and numbers of lesions per half-leaf and usually also increased numbers per unit area, whereas nitrogen consistently reduced numbers of lesions both per half-leaf and per unit area, and there was usually also a positive interaction between nitrogen and phosphorus both on leaf area and numbers of lesions.

Results qualitatively similar to those shown in Tables 3 and 4 have regularly been obtained when tobacco or *N. glutinosa* plants have been grown in a potting medium

containing soil, but not when plants have been grown in sand and watered with nutrient solutions. In these experiments all the nutrients were added in constant amounts except that either nitrogen or phosphorus was varied over a wide range. Table 5 shows the results of three such experiments in which nitrogen was given at three

Table 5. *The effects of varying nitrogen supply to plants grown in sand*

	Exp. 1. Tobacco			Exp. 2. Tobacco			Exp. 3. *Nicotiana glutinosa*		
N (mg.) applied twice weekly	Mean lesions per half-leaf	Mean lesions per sq.cm.	Mean wet weight of plants (g.)	Mean lesions per half-leaf	Mean lesions per sq.cm.	Mean wet weight of plants (g.)	Mean lesions per half-leaf	Mean lesions per sq.cm.	Mean wet weight of plants (g.)
1	8	0·34	8·7	13	0·54	11·3	10	1·09	6·4
20	408	6·37	31·0	68	0·99	38·0	71	2·42	23·0
200	4	0·42	4·8	30	0·93	9·5	3	0·34	7·5

The nutrient solution was applied twenty times in Exp. 1 and thirteen times in Exps. 2 and 3; except for nitrogen, all nutrients were kept constant.

different levels, 1, 20 or 200 mg. being given as $Ca(NO_3)_2$ twice weekly. There were ten plants for each level of nitrogen, three comparable leaves on each tobacco plant were inoculated with tomato aucuba mosaic virus and five leaves on each *N. glutinosa* plant were inoculated with tobacco mosaic virus. Opposite half leaves were inoculated with infective sap at different dilutions, but as the results were not qualitatively different, only results with sap at 1/500 are given. With both hosts, increasing the nitrogen supplied at each application from 1 to 20 mg. increased growth and numbers of lesions, both per half-leaf and per unit area, whereas increasing it to 200 mg. reduced growth and lesions. Other experiments suggested that the reduction in numbers of lesions occurs at nitrogen levels lower than those that retard growth. For example, in an experiment with tobacco plants and tomato aucuba mosaic virus, in which 1, 25 and 125 mg. of nitrogen were given twice a week, the average wet weights of the plants were 4, 47 and 56 g. respectively, whereas the average numbers of lesions per half-leaf were 281, 2455 and 181 and per sq.cm. 0·89, 1·01 and 0·11. Thus it would seem that, when supplied with adequate amounts of other nutrients, the effects of nitrogen depend on the amounts supplied. At low levels, it increases susceptibility proportionally more than it increases plant growth, whereas in medium amounts it reduces susceptibility while still stimulating growth, and at high levels it reduces both susceptibility and plant growth.

Table 6 shows the results of two experiments with tobacco plants grown in sand and supplied with various amounts of phosphorus in the form of KH_2PO_4, while other nutrients were kept constant. Over the range tested, variations in phosphorus had little effect on either plant growth or numbers of lesions produced by tomato aucuba mosaic virus, except that the highest level of phosphorus reduced both in Exp. 2.

TABLE 6. *The effects of varying phosphorus supplied to tobacco plants grown in sand*

P (mg.) applied twice weekly	Exp. 1			Exp. 2		
	Mean lesions per half-leaf	Mean lesions per sq.cm.	Mean wet weight of plants (g.)	Mean lesions per half-leaf	Mean lesions per sq.cm.	Mean wet weight of plants (g.)
1	20	0·20	38	68	1·35	19
5	18	0·17	47	52	0·85	24
25	22	0·21	48	46	0·79	22
125	27	0·29	41	12	0·29	14

The nutrient solution was given eleven times in Exp. 1 and thirteen times in Exp. 2; except for phosphorus, all nutrients were kept constant.

DISCUSSION

The obvious point that calls for discussion is the apparent difference in results obtained depending on whether plants were grown in sand or in mixtures containing soil. The most likely explanation of the discrepancy is suggested by comparing the chemical constitution of the plants grown in the different media. The phosphorus contents of plants grown in soil varied from a minimum of 0·09% of the total dry matter to a maximum of 0·52%, with a mean for all the treatments at 0·24% (Holden & Tracey, 1948). This compares with a phosphorus content of 0·43% of the total dry matter in the plants grown in sand when receiving the minimum amount applied (1 mg. twice a week) and with contents exceeding 0·75% of the dry matter with the larger applications. In other words, the maximum with plants in soil was approximately the same as the minimum with plants in sand. In the experiments with soil, it seems that most of the added phosphorus was rendered unavailable to the plants, and the amounts available with the different treatments covered a critical range in which both growth and susceptibility were influenced by phosphorus. The maximum needed for this response is apparently less than 2 mg. of available phosphorus per week, for amounts above this increased neither growth nor susceptibility. Similarly, with nitrogen, it seems that there is a critical range over which both growth and susceptibility are increased, and that this range was encountered with the smaller amounts of nitrogen supplied to plants grown in sand (Table 5), but not with plants grown in soil, where nitrogen was above this critical level, except in the presence of additional phosphorus. The nitrogen content of plants grown in soil varied from 1·03 to 6·89% of their dry matter (Holden & Tracey, 1948), whereas with those grown in sand it was 0·7, 4·63 and 6% for those receiving 2, 20 and 200 mg. of nitrogen twice weekly. No sand cultures were made in which potassium was varied, but there may be levels at which this element also affects both growth and susceptibility; its apparent lack of effect with plants grown in soil probably reflects the fact that the soil contained more than the necessary amount before supplements were added.

The main conclusion from this work seems to be that, in general, nutritional conditions most favourable to plant growth are also those that increase susceptibility. There would appear to be no one element that specifically encourages susceptibility, and we obtained no evidence to support Spencer's (1935) conclusion that nitrogen increased susceptibility when applied in amounts that retarded growth. It is clear that the balance between different nutrients is as important in affecting susceptibility as in affecting growth, and that the effect of adding a given quantity of one nutrient depends greatly on the availability of others. In the presence of adequate available phosphorus, for example, the addition of nitrogen may increase susceptibility and growth, whereas in its absence, the same addition may reduce both. An acute deficiency or a gross excess of any nutrient reduces susceptibility, and a balanced nutrient supply that gives optimal growth also approximates to the conditions most favourable for contracting virus diseases. With conditions obtaining in most field crops, it seems probable that varying nutrition will mainly influence the likelihood of becoming infected by changing plant size, but it also seems that, at certain critical ratios of different nutrients, variations may have effects additional to those on size. We may define these as changes in the intrinsic susceptibility of leaf cells, and with the tobacco mosaic viruses they are indicated by variations in the number of local lesions per unit area. Increases in intrinsic susceptibility were produced on occasions by increases in either nitrogen or phosphorus, depending on the amounts already present and their ratio to other nutrients. Except when correcting a gross unbalance, however, the changes in intrinsic susceptibility produced by varying nutrition were small compared with those produced by changing the light intensity under which plants are grown (Bawden & Roberts, 1947, 1948). Such differences in intrinsic susceptibility most likely do not result merely from changes in the epidermal cells that determine their resistance to wounding, for some differences were found both with mechanically transmitted viruses and with potato virus Y transmitted by aphids, but there is no evidence about the underlying mechanism.

We wish to thank members of the Statistics Department of Rothamsted for analysing the results statistically and Miss Margaret Holden for making the chemical analyses of the plants.

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Fig. 4

Fig. 3

Fig. 1

Fig. 2

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EXPLANATION OF PLATE 6

Comparable leaves from differently fertilized tobacco plants grown in a soil-sand-peat mixture, photographed 10 days after inoculation with tomato aucuba mosaic virus; Fig. 1, potassium only; Fig. 2, nitrogen and potassium; Fig. 3, phosphorus and potassium; and Fig. 4, nitrogen, phosphorus and potassium.

(*Received* 12 *July* 1949)

THE CORRELATION OF APHID NUMBERS WITH THE SPREAD OF LEAF ROLL AND RUGOSE MOSAIC IN POTATO CROPS

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(With 2 Text-figures)

An analysis of the results of experiments in different parts of England and Wales from 1941 to 1947 on the spread of potato leaf roll and rugose mosaic showed that leaf roll spread was correlated with the number of alate *Myzus persicae* (Sulzer) caught on sticky traps throughout the potato-growing season; there was some correlation with the maximum count of *M. persicae* per 100 leaves, but this possibly results from the correlation between trapped aphids and the number per 100 leaves. Spread of rugose mosaic (potato virus Y) was correlated to a lesser degree with number of *M. persicae*, perhaps because other aphid species are often vectors. With both diseases higher correlations were obtained when the infected plants were dispersed among the healthy crop than when they were placed together in a row. It is concluded that it is possible to predict the average health of potato stocks in the following year from average trap data; further work may enable the health of individual stocks to be predicted.

In the seed-potato areas of the north and west of the British Isles it is generally agreed that the extent to which leaf roll and rugose mosaic spread depends upon the size of the aphid infestation, particularly of *Myzus persicae* (Sulzer). Davies (1934) found in North Wales that the health of seed stocks was readily maintained when the maximum number of *M. persicae* did not exceed twenty per 100 leaves, but not when it exceeded 100 per 100 leaves. Murphy & Loughnane (1937) in Ireland, and Whitehead (1943) in Wales, also stated that there was a relationship between aphid populations on the plants during the summer and spread of virus disease, but did not express the relationship quantitatively. In the ware-growing districts of England, Doncaster & Gregory (1948) found that there was more spread both of leaf roll and rugose mosaic in years of heavy infestations of *M. persicae* than in others, but there was no simple relationship between aphid numbers on different potato crops and the spread of virus diseases. Broadbent & Gregory (1948) and Gregory & Read (1949) continued the work in the ware-growing areas. The accumulation of data from many fields now makes it possible to examine the relationships existing between numbers of apterous and alate aphids and spread of disease. For the first time trap catches of aphids are shown to be more closely related to spread of virus than are counts of apterous aphids on the plants.

Methods

Records of the spread of leaf roll and rugose mosaic (potato virus Y) were obtained in potato fields (mostly of the variety Majestic) in different parts of England and Wales during the years 1941–7 (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948; Gregory & Read, 1949), by taking tuber samples from the five plants in the row on each side of virus-infected plants which were dispersed or in a double strip across the rows. Counts of aphids, expressed as number per 100 leaves, are available for sixty of these fields and in thirty-three the numbers of aphids caught on sticky traps from May to September, during the growth season of the potatoes, were recorded.

As the plants near the infectors probably received more multiple infection than those some distance away, the mean percentage infection in the five plants on each side of the infected plants was transformed by the 'multiple infection transformation' (Gregory, 1948) to the estimated mean number of infective punctures made by the aphids per 100 plants ($\hat{I}$). The counts of aphids per 100 leaves varied within wide limits; the data were 'smoothed' by using the logarithms of the maximum counts. Counts of aphids throughout the season were not available for all fields, so for each the count made at about the maximum of the infestation was used. The Rothamsted roguing experiments are considered first separately (Table 1) as they were the only

Table 1. *Correlation of virus-disease spread with aphid data, Rothamsted roguing experiments*, 1943–7

(LR = leaf roll; RM = rugose mosaic.)

Infection	Aphid data	Correlation coefficient ($n=5$)	P	Regression coefficient
$\hat{I}$ (LR)	Trapped *Myzus persicae*	0·997	$<0·001$	$0·426 \pm 0·020$
$\hat{I}$ (LR)	Log maximum count	0·629	$<0·2$	—
$\hat{I}$ (RM)	Trapped *M. persicae*	0·181	$<0·8$	—
$\hat{I}$ (3) (RM)	Trapped *M. persicae*	0·585	$<0·3$	—
$\hat{I}$ (RM)	Log maximum count	0·776	$<0·05$	$9·348 \pm 4·393$
$\hat{I}$ (3) (RM)	Log maximum count	0·862	$<0·02$	$14·534 \pm 4·929$
$\hat{I}$ (3) (RM)	1943–6 trapped *M. persicae*	0·911	$<0·02$	$0·193 \pm 0·062$ ($n=4$)

experiments with dispersed infectors carried out over a number of years at one place. In this series no higher correlation was obtained by using 'aphid-days' as an expression of the population of aphids present throughout the season.

From 1943 to 1945 the sticky traps used were 3 ft. long (Doncaster & Gregory, 1948); in 1946–7, however, they were only 1 ft. long (Broadbent, Doncaster, Hull & Watson, 1948). Catches during the last 2 years were therefore multiplied by three, though this may have introduced a small source of error. The traps are thought to give a comparison between places of the relative numbers of aphids flying (Broadbent, 1948), though in the absence of wind-speed records no estimates of the absolute numbers can be made.

ROTHAMSTED ROGUING EXPERIMENTS, 1943–7

Table 1 gives the correlation and regression coefficients for $\bar{I}$ (estimated mean infection in the first five plants adjacent to the infective source) with the number of trapped *M. persicae* and with the logarithm of the maximum count of *M. persicae* per 100 leaves. Spread of leaf roll is seen to be highly correlated with the number of alate *M. persicae* trapped (Figs. 1 and 2), but less so with log maximum count (Fig. 1). Spread of rugose mosaic within five plants ($\bar{I}$) or three plants ($\bar{I}(3)$), for

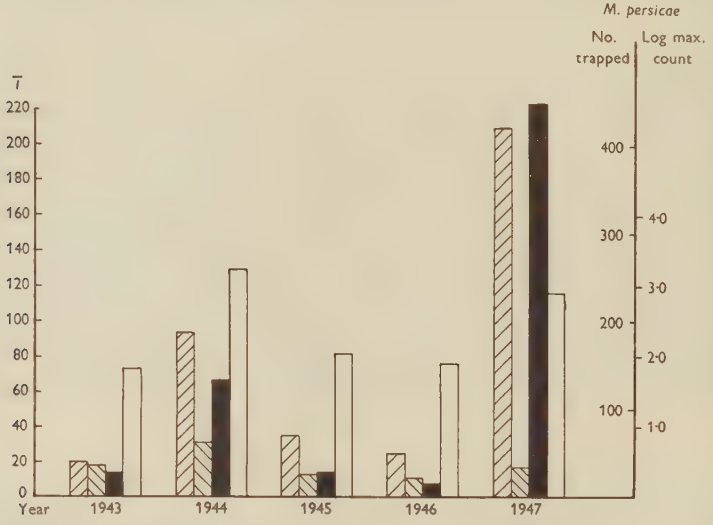

Fig. 1. Rothamsted roguing trial results, 1943–7. ▨ Leaf roll spread (I). ▧ Rugose mosaic spread ($\bar{I}$). ■ Trapped *M. persicae*. □ Log max. count of *M. persicae* per 100 leaves.

the five years is correlated with log maximum count, but is not correlated with trapped *M. persicae*; for the four years 1943–6, however, it is correlated with trapped *M. persicae*. In these years spread was largely from infected plants within the crop, whereas in 1947 large numbers of *M. persicae* reached the potatoes in mid-July and apparently introduced leaf roll but not rugose mosaic. Rugose mosaic plants are often small and die early, but correlation is not increased by relating spread with trap catches for May–July, May–August, or with the sum of fortnightly counts on the crop.

LEAF-ROLL INFECTION IN ALL TRIALS

The Rothamsted roguing experiments gave data on spread of infection from single diseased plants, as did results from commercial fields (Doncaster & Gregory, 1948). From other experiments (Doncaster & Gregory 1948; Broadbent & Gregory, 1948) information was obtained on spread from a double strip of infected plants. Table 2

TABLE 2. *Correlation of leaf roll spread* ($\bar{I}$) *with aphid data*

(S = Sutton Bonington, 1945.)

	No. of trials	Correlation coefficient	P	Regression coefficient
Trapped *M. persicae*				
All trials	33	0·452	$<0·01$	$0·099 \pm 0·035$
All trials, omitting S	32	0·849	$<0·001$	$0·351 \pm 0·040$
Trials with single infectors	17	0·952	$<0·001$	$0·445 \pm 0·037$
Trials with a strip of infectors, omitting S	15	0·581	$<0·02$	$0·152 \pm 0·059$
Log maximum count				
All trials	60	0·555	$<0·001$	$30·142 \pm 5·932$
All trials, omitting S	59	0·589	$<0·001$	$34·268 \pm 6·225$
Trials with traps	33	0·501	$<0·01$	$28·707 \pm 8·910$
Trials with traps, omitting S	32	0·568	$<0·001$	$36·992 \pm 9·784$

presents the correlation and regression coefficients for $\bar{I}$ on trapped *M. persicae* and on log maximum count, both including and omitting the data from Sutton Bonington, 1945. In 1945 Sutton Bonington was exceptional in that the aphid count of about 8000 *M. persicae* per 100 leaves and trap catch of 892 were both twice as great as counts recorded at any other site in any year, but the spread of disease was not in proportion.

Leaf roll spread is highly correlated with trapped *M. persicae* and with log maximum count, which are correlated with each other. The correlation coefficient of trapped *M. persicae* with log maximum count = 0·678, $P < 0·001$; the regression coefficient = $106·810 \pm 21·170$.

A multiple regression of leaf roll spread ($\bar{I}$) on trapped *M. persicae* and on log maximum count (thirty-two trials) gives:

	Partial correlation coefficient	P	Partial regression coefficient
On trapped *M. persicae* (eliminating log max. count)	0·767	$<0·001$	$0·355 \pm 0·055$
On log max. count (eliminating trapped *M. persicae*)	−0·021	$>0·9$	$-0·916 \pm 8·681$

Thus, if log maximum count is held steady, variations in trapped *M. persicae* are correlated with variations in spread of leaf roll; but if trapped *M. persicae* are held steady, variations in log maximum count are not correlated with variations in spread of leaf roll.

The spread of leaf roll from dispersed diseased plants is more highly correlated with trapped *M. persicae* (Fig. 2) than is that from a strip of infectors (Table 2). The coefficients indicate that the regression lines for leaf roll spread on either trapped *M. persicae* or log maximum count are much steeper than the corresponding lines for rugose mosaic infection. This conforms with the fact that leaf roll usually increases more than rugose mosaic in the variety Majestic. The regression lines for

leaf roll infection on log maximum count (all trials, and all trials with traps, omitting Sutton Bonington, 1945) cut the axis at log maximum count = 1·24 and 1·34 respectively, indicating that leaf roll would probably not spread with maximum counts below 17–22 aphids per 100 leaves, which is in close agreement with the figure of 20 suggested by Davies (1934).

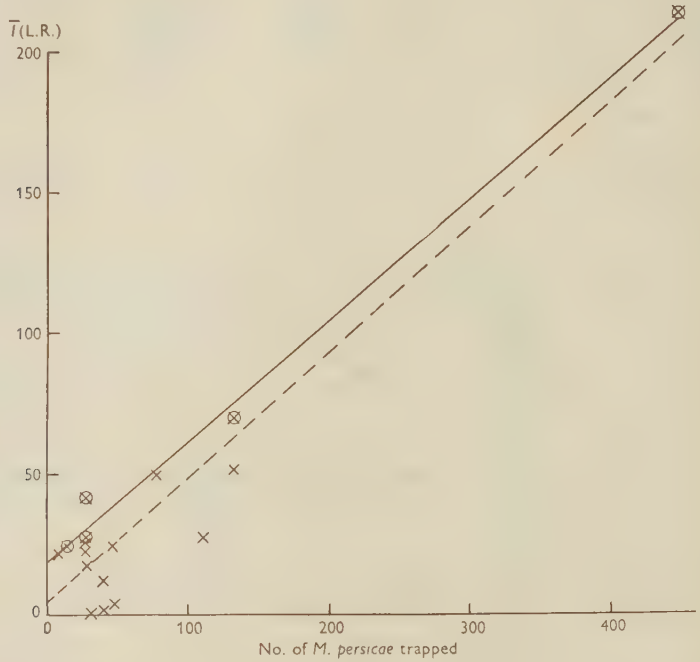

Fig. 2. Regression of leaf roll spread ($\bar{I}$) on number of *M. persicae* trapped.
○——— Rothamsted roguing trials.
×– – – All trials with single infectors.

Rugose mosaic infection in all trials

Fewer data are available for rugose mosaic than for leaf roll; in forty-six trials (Table 3) there is no correlation with log maximum count. There are two sites at least—Sutton Bonington, 1945 and Rothamsted, 1947—where trap catches are exceptional for reasons stated above. If these are omitted, there remain twenty-four trials with traps in which there is a correlation between rugose mosaic infection and trapped *M. persicae* ($P < 0{\cdot}05$). In ten trials with dispersed infectors (omitting Rothamsted, 1947) infection is correlated with trapped *M. persicae*; there is no correlation in the fourteen trials with strips of infectors.

Macrosiphum euphorbiae (Thomas) (= *M. solanifolii* Ash.) is known to be a vector of potato virus Y in the laboratory, but adding the number trapped to the number of

Myzus persicae does not significantly increase the correlation (Table 3). The identification of *Aphis rhamni* (Fonsc.) from trapped specimens is not considered sufficiently reliable for this species to be included.

TABLE 3. *Correlation of rugose mosaic spread* ($\check{I}$) *with aphid data*

(S = Sutton Bonington, 1945; R = Rothamsted, 1947.)

	No. of trials	Correlation coefficient	P	Regression coefficient
Trapped *Myzus persicae*				
All trials, omitting S	25	0·320	$<0·1$	—
All trials, omitting S and R	24	0·405	$<0·05$	$0·052 \pm 0·025$
Trials with single infectors	11	0·359	$<0·3$	—
Trials with single infectors, omitting R	10	0·805	$<0·01$	$0·145 \pm 0·038$
Trials with a strip of infectors, omitting S	14	0·261	$<0·4$	—
+ *Macrosiphum euphorbiae*, all trials, omitting S and R	24	0·464	$<0·02$	$0·055 \pm 0·022$
Log maximum count				
All trials, omitting S	46	0·201	$<0·2$	—

DISCUSSION

The experiments quoted showed that there can be large differences between the spread of virus diseases in potato crops carrying aphid populations of similar size, whereas similar spread sometimes occurs with widely differing aphid populations. Nevertheless, when results from a number of crops are averaged there is a general correlation between the spread of virus and aphid numbers, especially with trapped *Myzus persicae* in the experiments in which the infectors were dispersed.

The errors of the regression coefficients give an indication of the accuracy to be expected from field trials with sticky traps. In Table 1 the standard error is 4·7% of the regression coefficient of spread of leaf roll on trapped *M. persicae*. In Table 2 the standard error is 8·3% of the regression coefficient of spread of leaf roll from single infected plants on trapped *M. persicae*, and 39% with spread from strips of infected plants. The standard errors expressed as percentages of the regression coefficients of spread of leaf roll on log maximum count vary from 18 to 31% (Table 2). Thus trapped *M. persicae* not only give higher correlation coefficients than log maximum count, but also a smaller standard error of the regression coefficient.

The correlation of spread of rugose mosaic (Table 3) with trapped *M. persicae* or log maximum count is not so high as that of leaf roll. It is possible that aphids other than *M. persicae* often spread rugose mosaic, but not leaf roll.

It has often been assumed that virus diseases are brought into the crop from a distance by alate aphids and that most of the subsequent spread within the crop is the work of apterae, but recent work (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948) has suggested that alatae probably play a large part in disseminating potato viruses within the crop and that much of the spread takes place early in the

season. But during this period few *M. persicae* are trapped; most are caught during the period of maximum infestation of the potatoes (usually July–August), when a large proportion develop wings, and leave the crop, causing a rapid decline in numbers. The correlation between trapped *M. persicae* and log maximum count suggests that most of the aphids trapped in a field are those which are leaving the crop; the number trapped early in the season is usually very small. It is not clear why spread of disease is correlated with aphids trapped after much of the spread has occurred, unless there is a constant ratio between the number of colonizing alatae, which spread much virus, and the number of alatae leaving the crop during the summer. It is not usual for large numbers of alate *M. persicae* to colonize potatoes in the British Isles; the numbers arriving on the young crop are invariably small, and small numbers no doubt continue to colonize the potatoes throughout the season. But occasionally, as at Rothamsted in 1947, very large numbers colonize a well-grown crop; the evidence suggested that these aphids were carrying leaf roll virus, but not potato virus Y and that when such large numbers visit a crop late in the season there will be no resultant correlation with virus spread unless the aphids bring the virus with them.

The regression line for the seventeen trials with dispersed infectors (Fig. 2) cannot be relied upon for forecasting, as the experimental conditions varied and there are insufficient trials with large numbers of aphids (the seasonal catches of *M. persicae* were all under 150, except at Rothamsted in 1947). However, the regression line for these seventeen trials is almost parallel to that for the five Rothamsted roguing trials, which were made under conditions approximately standard; this suggests that the slope of the lines might give an indication of the average relation of aphid numbers to spread of leaf roll (Table 4).

TABLE 4. *Increase in $\bar{I}$ (Rothamsted roguing trials) and equivalent increases in percentage infection of leaf roll for increases of fifty aphids*

Increase in trapped *Myzus persicae*	Increase in $\bar{I}$ (LR)	Equivalent percentage increase
From 50 to 100	22	13
From 100 to 150	22	11
From 150 to 200	22	8

One of the objects of potato virus field studies involving aphid surveys has been to determine if it is possible to predict, from data obtained, the health of stocks in the following year. The present paper shows that it is possible to predict, with a high degree of accuracy, the *average* health in the following year from *average* trap data, if the health in the current year is known. This is a contribution to the ultimate aim of predicting the health of individual stocks. Hauschild (1947) derived theoretically a formula by means of which the health of a crop at lifting could be estimated, knowing the initial health and the course of the aphid infestation; the German workers hope to use such a formula in forecasting the amount of virus spread. If further

work confirms that alatae are much more important than apterae in spreading virus, traps might give better results than counts on the plants, but sticky traps have not been tested in good seed-growing areas, where aphids are usually few. Trials on a uniform plan with dispersed infectors and traps will show if the health of individual stocks can be predicted with accuracy. Having determined the regression from such trials, abnormal fields can be recognized and data from them can be studied to find what further factors, or methods, need consideration.

Grateful acknowledgement is made to members of the Statistics Department, Rothamsted Experimental Station, for checking the calculations.

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(*Received* 18 *July* 1949)

STUDIES ON *LUCILIA* SPECIES (DIPTERA) UNDER DANISH CONDITIONS

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(With 2 Text-figures)

Whilst sheep myiasis caused by *Lucilia sericata* is a severe economic problem in Great Britain, it rarely reaches such proportions in countries bordering Western Europe. Possible reasons for this relative absence of myiasis were investigated in Denmark.

During the period 19 July–15 August 1948, trapping studies showed that blowflies normally associated with myiasis in Great Britain, and particularly *L. sericata*, occurred fairly abundantly in the Mols area of Jutland.

The reactions of Danish *L. sericata* to attractive materials placed on sheep were similar to those shown by the species under British conditions. The number of egg batches laid on sheep in response to chemical stimuli were fewer than would have been expected in Great Britain.

Studies on the fleece microclimate showed that relative humidities of 50% or more frequently occurred on the skin surface. Humidities 3 cm. above the skin were markedly lower. Except during or immediately after rain, humidities high enough to ensure the hatching of blowfly eggs were never recorded.

It is concluded that the absence of myiasis in Denmark and its relatively low incidence in other countries is not due to the existence on the Continent of a strain of *L. sericata* differing in oviposition habits from the British species.

Lucilia sericata (Mg.) which is responsible for approximately 96% of sheep myiasis in Great Britain (MacLeod, 1943) is a cosmopolitan species known from practically all sheep-farming areas of the world. There are, however, several regions in which sheep are farmed where the species occurs without causing myiasis, or where myiasis rarely becomes an economic problem.

Even in Western Europe where the species is indigenous the incidence of myiasis varies considerably. Recent information on the position is summarized in Table 1. Compared with Great Britain, countries bordering Western Europe are remarkably free from myiasis. Myiasis occurs very infrequently in France, Belgium and Denmark. In Sweden, isolated outbreaks occur. In some districts of Norway myiasis sometimes causes considerable losses. In Holland, myiasis usually occurs infrequently, but there are several records of severe outbreaks. Bos (1887*a*,*b*) recorded losses in 1875, and quoted earlier work suggesting that the first outbreak in Holland was in 1860. It was then alleged that the disease had been imported into Holland with English sheep. Bos, however, related its occurrence to the presence of scouring

* The field investigations were made at the Mols Laboratory for Terrestrial Ecology, Femmøller, Jutland.

sheep. This condition was associated with rich grazings and marshy areas but was absent from the sandy and heath lands. Bos thought that myiasis arose from a minor change in the egg-laying habits of the fly, which he regarded as a carrion and dung-breeding species. Thus, from laying its eggs on dung, the fly developed the habit of laying its eggs on wool coated with dung. Karsch (1887) challenged the view that *L. sericata* could breed in dung. Recent work supports Karsch, although Thomsen

TABLE 1. *Information on the occurrence of myiasis in Western Europe*

Country	Sheep population	Density per sq. mile	Occurrence of myiasis
Belgium	170,000 (1947)	15	Occasional records of infested wounds but no true cutaneous myiasis
Denmark	213,431 (1945)		Two cases noted approximately 15 years ago and
	85,640 (1947)	5	one reported in June 1948.*
France	9,872,370 (1939)		Occasional cases but never of economic impor-
	7,258,942 (1946)	34	tance
Germany (Western Zone)	2,490,916 (1948)	18	Myiasis rarely occurs
Germany (Eastern Zone)	780,000 (1948)		
Netherlands	689,501 (1939)		Occasional cases but not usually of economic im-
	424,859 (1948)	32	portance. 288 cases in a flock of 1200 sheep in 1932. (For other records of myiasis see Intro- duction)
Norway	1,744,000 (1939)		Myiasis occurs in Norway, particularly in western
	1,225,000 (1947)	10	areas where, in certain years, it may lead to quite considerable losses. The south-western and western areas are the main sheep-farming districts
Sweden	519,820 (1943)		Myiasis is not usually of economic importance.
	420,774 (1947)	2½	During the period 1943–9 one severe outbreak was reported in Scania
England and Wales	17,986,000 (1939)		Myiasis occurs generally throughout the British
	10,858,000 (1948)	187	Isles
Scotland	8,007,134 (1939)		
	6,730,664 (1948)	226	,, ,,
Ireland	3,942,442 (1939)		
	2,632,502 (1948)	82	,, ,,

The data on sheep populations and on the occurrence of myiasis were obtained mainly through the London embassies of the continental countries included in the survey. In some cases information was obtained direct from sources within those countries. As a very approximate index of sheep density the number per square mile is given.

(1938) recorded an isolated instance of *L. sericata* breeding in pig manure. It is interesting to note that the most recent outbreak in Holland, reported by Baudet & Nieschulz (1933), occurred on a freshly established polder where the grassland was rich and where scouring occurred. Sheep on old grazings in the neighbourhood were free from attack.

The reasons for the infrequent occurrence of myiasis in European countries,

* In December 1949, the writer received information of another slight case of myiasis in Denmark during late August 1948.

where conditions are not apparently very different from those occurring in some parts of Great Britain, have never been determined. In order to provide some information on this problem the following investigations were carried out in Denmark. The results obtained obviously have a bearing on the problem for other parts of Europe.

Notes on the area and weather conditions

All the Danish field observations were made on the Mols peninsula some 20 km. north-east of Aarhus, Jutland. The area has been described in detail by Thamdrup (1946–7). It contains some of the highest land in Denmark, the so-called Mols

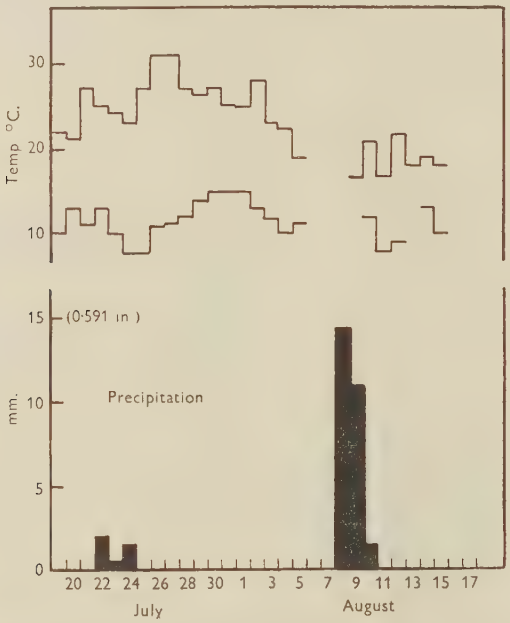

Fig. 1. Precipitation and shade max., min., temperatures (screen height) recorded at Mols during the period of the investigations. Temperature readings 19–30 July were recorded near the laboratories. From 31 July to 16 August the readings refer to the heathland where the sheep were grazed.

mountains of moraine origin (highest point 244 ft. above sea-level). For the most part the soils are of sand and gravel, but areas rich in humus occur on the plain between the high land and the sea. The country, with its steep slopes and varied flora, gives a wide range of animal habitats.

The field observations were made from 19 July to 15 August 1948 inclusive. Temperatures and precipitation for this period are shown in Fig. 1. The weather, in general, was dry and hot with several hours of bright sunshine on most days.

Warm showery periods alternating with sunshine and high atmospheric humidities, which in Great Britain are usually considered ideal for the development of blowfly strike, did not occur at Mols during the period of the observations. The sheep used for the experiments were grazed on a dry, sandy, pasture of poor feeding value. Thus neither the weather nor pasture conditions were favourable for the occurrence of myiasis.

Sheep farming in Denmark

In order to determine whether there were any features in the management of Danish sheep which might account for the absence of myiasis, information was obtained from Konsulent H. Nislev, adviser to the Danish Sheep Breeders' Association (Faellesudvalget til Faareavlens Fremme). Konsulent Nislev also gave the writer a personally conducted tour of sheep farms in various parts of Denmark.

The greater part of the sheep population is kept in buildings from December to March and the ewes are clipped twice in the course of the season. Lambing practice varies. In some flocks the lambs are born and reared in buildings and are sold for meat at Easter. In others, the lambing may take place in buildings but the lambs are turned out shortly after birth. Whilst such practices would tend to lessen the chances of myiasis among ewes, they could not possibly reduce the chances of free-range lambs becoming infested. Furthermore, on the west coast of Jutland where some 10,000 marsh sheep are grazed the ewes are clipped only once per year. They remain outside throughout the winter, lambing occurs outside and the flocks are managed on lines similar to those used in some parts of Great Britain.

Dipping, as practised in Great Britain, plays no part in Danish sheep husbandry. The sheep are usually washed before shearing and if necessary hand-treated against scab (*Psoroptes communis ovis*) and lice (*Haematopinus ovis*).

Breed of sheep and wool type cannot be responsible for the absence of myiasis. The main sheep breeds are Oxford Down and Leicester, neither of which is free from myiasis in Great Britain. Furthermore, in both breeds, rams and ewes have often been imported from England.

On the whole, flocks are small. Where there are less than twenty ewes the animals are usually tethered. Large flocks are on free range; the largest flock numbered approximately 800 sheep.

Apart from the possible importance of a low host density, it would seem that methods of sheep management in Denmark are not sufficiently different from those practised in Great Britain to influence the chances of myiasis.

The relative abundance of *Lucilia* species

In order to determine the relative abundance of blowflies in the Mols area, six mosquito netting traps of the type used in other blowfly investigations (Cragg & Ramage, 1945; Cragg & Thurston, 1950) were erected at the following positions:

Trap 1 (Thamdrup, 1946–7; map ref. E 1) was situated at the boundary of an oat

field and a meadow on the bank of a small stream. Cattle were grazed in the meadow throughout the period of the investigations. Beyond the stream and some 20 yd. to the south-west of the trap was dense beech wood. The trap, whilst in an open situation, did not receive full illumination when the sun was at its zenith.

Trap 2 (Thamdrup, 1946–7; map ref. E2) was placed in open oak scrub which passed on its southern side into a *Calluna*-covered slope. The trap received little illumination during the early part of the day and only for short periods did it receive direct illumination.

Trap 3 (Thamdrup, 1946–7; map ref. H6) was situated on an open *Calluna*-covered slope on the northern side of a Juniper bush. It received direct illumination from the west and south-west.

Trap 4 (Thamdrup, 1946–7; map ref. J6) was situated on an exposed heather moor with patchy growths of lichen, *Avena pratensis*, *Festuca rubra* and *Agrostis tenuis*. It was on this moor that the sheep used in the investigation were grazed. This trap received full illumination throughout the day.

Trap 5 was situated in a shallow trench on the moor referred to under trap 4. The trench gave it some protection from the wind and the amount of illumination it received was little different from that of trap 4.

Trap 6 was also situated on the moor (see trap 4). It lay some 20 ft. to the south of the sheep pen and on the southern aspect of a Juniper bush. It received full illumination throughout the day.

The trapping results are summarized in Table 2. The general distribution of the main species is in keeping with unpublished data obtained in North Wales and Durham. Trap 2, which received little direct sunlight, showed the highest *Lucilia caesar* (L.)/*L. illustris* (Mg.) catches. *L. silvarum* (Mg.) occurred on the outskirts of woodland and *L. sericata* (Mg.) predominated in very open situations. Of special interest was the high percentage of *L. pilosiventris* Kram., caught by traps 4 and 5 on the open heathland. This species does not occur in Britain. *L. richardsi* Coll., a species closely related to *L. pilosiventris* and which occurs in Britain (Aubertin, 1933), was not found. Traps baited with ethyl mercaptan—hydrogen sulphide mixture (Cragg & Thurston, 1949) showed a degree of specificity similar to that described in connexion with its use in Great Britain. Thus when the mixture was used in trap 2, *Calliphora vomitoria* (L.) was rarely captured, although previously it had formed a high percentage of the catches. The disappearance was not due to a marked reduction in the *C. vomitoria* population because many *C. vomitoria* quickly settled on meat exposed in the vicinity of the ethyl mercaptan—hydrogen sulphide trap.

Table 3 gives the combined daily catches of *Lucilia sericata* made by traps 4 and 6. For comparative purposes, the catches for two traps in operation at Durham are given. Whilst the Danish *L. sericata* catches compare favourably with those obtained under British conditions, such figures give no indication of the relative size of the two populations from which they were drawn.

TABLE 2. *Numbers of blowflies caught by different traps*

Trap no.		No. of days	*L. sericata* ♂♂	*L. sericata* ♀♀	*L. pilosiventris* ♂♂	*L. pilosiventris* ♀♀	*L. silvarum* ♂♂	*L. silvarum* ♀♀	*L. illustris* ♂♂	*L. ampullacea* ♂♂	*L. caesar* ♂♂	*L. caesar* group* ♀♀	*Calliphora vomitoria* ♂♂	*Calliphora vomitoria* ♀♀	*Calliphora erythrocephala* ♂♂	*Calliphora erythrocephala* ♀♀	*Protophormia terrae-novae* ♂♂	*Protophormia terrae-novae* ♀♀
A	1	21	0	10	0	0	5	32	12	1	18	183	0	1	1	8	0	1
	2	21	1	5	0	0	1	2	6	1	72	771	9	48	9	14	4	4
	3	14	0	33	No records		0	0	0	0	1	0	0	1	0	1	0	0
	4*a*	21	1	62	0	15	0	0	0	0	0	4	0	0	0	0	1	0
	4*b*	6	1	67	No records		0	0	1	0	0	8	0	0	0	0	0	0
	5	14	2	50	0	20	0	0	0	0	0	0	0	0	0	4	0	0
	6	21	1	43	0	4	0	0	0	0	0	13	0	0	0	0	0	0
B	1	6	0	5	0	0	0	0	0	0	0	152	0	0	0	0	0	0
	2	6	0	18	0	0	0	0	0	0	1	1349	0	5	0	0	0	0
	6	6	1	78	No records		0	0	0	0	0	7	0	0	0	0	0	0

In section A the traps were baited once per week with ½ lb. pig lung.
In section B they were baited once daily with 10 ml. 0·2 % ethyl mercaptan and 10 ml. saturated hydrogen sulphide.
The first trapping day for all readings in A, with the exception of trap 4*b*, was 20 July.
Section B readings and trap 4*b* were started on 10 August.

* '*L. caesar* group females' gives the total catch of females of *L. illustris*, *L. ampullacea* and *L. caesar*. These species can only be distinguished in the males.

TABLE 3. *The total daily catches of female* Lucilia sericata *in two traps at Mols, Denmark, compared with catches recorded at Durham, England*

	July											August														Total catch
	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Catches at Mols	0	2+	15	6	16	9	4	1	4	10	3	1	3	3	5	20	3	9	0	0	3	3	14	9	19	71	27	260
Catches at Durham	2	3	1	7	16	31	16	7	10	3	5	19	28	4	3	0	1	1	2	1	0	0	2	0	0	13	22	197

The Danish figures are the totals for traps 4 and 6 which were the two traps maintained in the vicinity of the sheep, and furthermore baited with meat throughout the period of the investigations. The traps at Durham, also baited with meat, were operated in the grounds of the Science Laboratories in close proximity to arable land. Throughout the period of the observations there were no sheep in their immediate vicinity.

Observations on carcasses

Before female *L. sericata* become gravid they must have two to three protein meals (Hobson, 1938). Hobson's laboratory experiments suggested that carrion was probably the only source of suitable protein under wild conditions. Thus the amount of carrion available might, in certain areas, be a limiting factor in the production of gravid females. During the period at Mols, no naturally occurring carrion was found on the heathland where the sheep were grazed. The following observations were made on shot animals.

Three squirrel carcasses were exposed under the different conditions described for traps 2, 3 and 4. In all cases the first colonizer was the ant *Formica pratensis*. At sites 3 and 4, they occurred in such numbers that they markedly restricted blowflies from either feeding or ovipositing. Observation periods of $\frac{1}{2}$–1 hr. were spent daily at each carcass during times when flies were active around the traps. In the case of carcasses '3' and '4' at the end of 4 days when under British conditions they should have been extensively colonized with blowfly larvae, only isolated larvae were present. The normal carrion beetles also colonized these carcasses so that out of the small number originally present few blowfly larvae could have possibly survived. Certainly no third instar *Lucilia* larvae were found on these carcasses and only isolated flies were ever observed trying to feed on them.

Carcass '2' whilst colonized by ants never swarmed with them but those present proved troublesome to flies which attempted to settle on the carcass. Because of its situation, *Calliphora vomitoria* and *Lucilia caesar* were the main blowflies seen on it. *L. sericata* was never observed on it. Some *Calliphora* larvae were able to complete their feeding stage on this carcass.

These observations suggest that at times when certain ant species are numerous, the opportunities for *Lucilia sericata* to obtain both food and breeding sites may be limited.

The number of observations is too small to warrant any emphasis being given to these results, but they indicate a problem well worth further attention. In Australia, Gilmour, Waterhouse & McIntyre (1946) showed that a high percentage of non-gravid females became gravid within 3–4 days of liberation. It should be remembered, however, that rabbits, which must form an important source of natural carrion in Australia, are absent from Denmark except on some small islands.

The reactions of *Lucilia sericata* to attractants placed on sheep

A total of six ewes tethered in pairs and five lambs were available for the experiments. This comprised the sheep population of the farm of approximately 370 acres (150 ha.). On English standards the sheep population of the Mols area was negligible. The sheep grazed on open heathland and the majority of the observations were made on lambs because the ewes had been shorn in late May and their fleeces were very short. The sheep were of Leicester body form with moderately slack fleeces.

The number of experiments made on sheep were kept as small as possible in view of the dangers associated with such experiments in an area where *L. sericata* was not established as a pest.

Hobson (1935, 1936) developed a method for inducing *L. sericata* to oviposit on sheep. His standard technique, which consisted of fastening a pad soaked in a solution of 2% ammonium carbonate and 0·04% indole in the fleece of a sheep, has proved an effective method of stimulating oviposition not only in Great Britain but also in Australia (Mackerras & Mackerras, 1944). Using the Hobson pad technique, Cragg (1950) has shown that under British conditions it is possible to differentiate between factors which attract flies to sheep and those which actually produce oviposition. Thus, given suitable climatic conditions, attraction can be brought about by placing pads of ethyl mercaptan on sheep, and the flies so attracted will oviposit on a pad of 0·1% ammonium carbonate if it is placed in the fleece near to the mercaptan pad.

TABLE 4. *Occurrence of oviposition on sheep*

	July						August	
	23	24	25	28	29	31	3	11
No. of sheep used	1	3	3	3	3	3	3	4
No. of sheep on which eggs were laid	0	2	1	2	1	1	1	3
No. of egg batches	—	1, 1	2	1, 2	1	1	1	1, 2, 1

On 11 August two pads, one containing 5 ml. 0·02% ethyl mercaptan and the other 5 ml. 0·1% ammonium carbonate were placed on all the sheep tested. In all other experiments a single pad containing 5 ml. 2% ammonium carbonate with 0·04% indole was used.

Experiments using these techniques were carried out at Mols and the results are given in Table 4. Oviposition was recorded in all the trials with the exception of the first in which only one lamb was used. The eggs laid in four of the trials were reared and yielded pure samples of *L. sericata*. In some of the trials oviposition by *L. sericata* was actually observed. It is clearly evident therefore that under Danish conditions *L. sericata* will respond to chemical substances known to provoke oviposition under British conditions. Furthermore, as the experiments on 11 August showed, the species responded to attractive materials and under such circumstances was induced to oviposit on a pad of 0·1% ammonium carbonate. In three cases it was possible to observe the searching activities of flies which subsequently oviposited on sheep. Their behaviour appeared to be similar to that shown under British conditions. In particular, when the flies finally settled near the pad they were not easily disturbed.

On no occasion were more than three flies observed to be active on any one sheep, but it was not possible to keep the sheep under continuous observation. The amount of oviposition which occurred was certainly less than would have been expected under British conditions.

On two occasions when eggs were laid on sheep, the eggs were not removed but

left in position under moist pads. The eggs hatched and the larvae established themselves on the skin.

It follows, from these observations, that given the right stimulus, Danish *L. sericata* will lay eggs on sheep and that such eggs can give rise to myiasis.

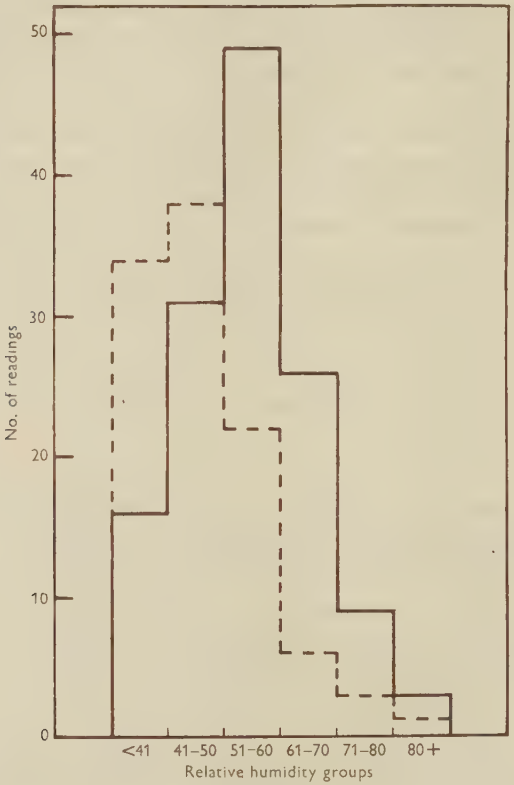

Fig. 2. Histograms based on the R.H. determinations obtained on three lambs during the period 20 July–14 August 1948. The readings were taken at intervals of 2 days at four positions on each animal. Readings obtained during continuous rain on 8 August are not included. Free moisture was present throughout the fleece and all twelve readings at each level were over 80% R.H. Broken lines show the histogram for the R.H. in the fleece 3 cm. above the skin surface. Continuous lines show the R.H. at the skin surface.

Humidity conditions in fleeces

As far as other investigations allowed, humidity determinations were obtained for one ewe and three lambs at 2-day intervals throughout the period 20 July–14 August. In addition to these data, supplementary information was obtained for a further two ewes and two lambs. Humidities were determined by using cobalt chloride

papers prepared according to Solomon (1945). Their use in studying the microclimate of the fleece has been fully described by Davies (1948*b*), and the same technique was followed in the present investigation. For the routine measurements, determinations were made at two levels, namely, skin surface and 3 cm. above the skin. Such readings were obtained for four positions: withers, mid-back, tail-head and left crutch. These positions were comparable with those similarly described by Davies (1948*b*).

All the readings for the three lambs are summarized in Fig. 1. Neglecting for the moment those readings obtained when the fleeces were wet to the touch either during or following rain, it will be seen that:

(1) Skin-surface humidities were generally higher than at 3 cm. above the skin. This was emphasized when the individual pairs of readings for each position were compared. Out of a total of 138 pairs in only twelve cases were the readings at 3 cm. higher than the base readings, and in a further twelve cases the readings were equal. There is little doubt, therefore, that the typical humidity gradient in these and in other sheep studied at this particular time, had its highest value near the skin surface.

(2) Approximately 65% of the skin surface readings were above 50% R.H.

Davies (1948*a*) has shown that a proportion of *L. sericata* eggs can complete development at 50% R.H. and 37° C. Thus, under Danish conditions humidities suitable for development were recorded fairly frequently at the skin surface. Often, the cobalt chloride papers, although only 1 cm. square, might show a gradient from 90–100% on the edge next to the skin to less than 50% on the edge away from the skin. Thus the high humidity zone might be less than 1 cm. in depth and sometimes was even less than 2 mm. Throughout the period of the investigations, humidities over 80% R.H., and therefore high enough to allow both successful hatching and early larval development of *L. sericata*, were only found during and immediately after heavy rain. In the case of each sheep the skin surface R.H. readings obtained for the four positions studied, were compared. Statistical analysis of the results showed that there was no significant difference between the R.H. readings obtained for the various positions. In two cases $P = 0{\cdot}1$, but in the majority of cases P was $> 0{\cdot}5$.

The direction of the humidity gradient (with the highest R.H. occurring next to the skin) does not conform with the general observations of Davies & Hobson (1935), MacLeod (1939) and Davies (1948*b*). A gradient of this type has, however, been recorded in certain special cases. Cragg & Davies (1947) showed it to be present in sheep subjected to severe muscular exercise. Also, Davies (1948*b*) found such a gradient in two Down-Cross lambs examined during a period of hot weather. In the latter case the skin R.H. was sufficiently high to allow *L. sericata* eggs to develop and hatch. Whilst the Danish sheep had Down-type fleeces there is no reason to suppose that the gradient pattern is a characteristic of such sheep, for MacLeod (1939) used Down sheep without recording this type of gradient. Further, at the time that Davies (1948*b*) noted the above results no comparable observations were

made for other breeds. On the available evidence it would appear that Davies's (1948*b*) results and the present results arose because high atmospheric temperatures caused the sheep to sweat and the resulting moisture was rapidly dispersed through the fleece.

Discussion

The following conditions must be fulfilled before myiasis is established:

(*a*) Sheep must become attractive to blowflies.

(*b*) Flies must react to attractive sheep.

(*c*) Gravid flies must occur in sufficient numbers to ensure that attractive sheep are found.

(*d*) Environmental conditions must be suitable for both attraction and oviposition.

(*e*) The microclimate of the fleece must provide conditions in which eggs can develop and hatch, and in which first-instar larvae can invade the skin of the host.

Under Danish conditions some *Lucilia sericata* were induced to oviposit on sheep. Thus, conditions (*b*), (*c*) and (*d*) existed in Denmark.

With regard to (*a*) the changes which make sheep susceptible to blowfly attack are inadequately understood. A major factor appears to be the formation in the living fleece of compounds often associated with putrefactive processes (Mackerras & Mackerras, 1944; Cragg & Ramage, 1945; Cragg, 1950). Susceptibility may be influenced by climatic and edaphic factors. At Mols, low rainfall, bright sunlight, thin pastures and very light soil would tend to keep the fleeces healthy and clean. But the soil conditions at Mols were not general for Denmark. In West Jutland, large numbers of sheep were grazing on coastal marshes. Further, scouring, which in Great Britain often gives rise to myiasis, occurs in Danish sheep. It would appear possible, therefore, that in some areas of Denmark, sheep might become attractive to blowflies.

If susceptible sheep did occur in Denmark and *L. sericata* oviposited on them, then the development of myiasis would be dependent on (*e*) the state of the fleece microclimate.

Even in Great Britain fleece humidity is an important limiting factor in the production of myiasis. This was emphasized by Davies & Hobson (1935), and their general conclusion has been substantiated by other investigations. MacLeod (1937) recorded that of sixty-four egg masses found on a flock, only twenty-nine gave rise to myiasis, in spite of apparently very favourable conditions. Davies (1948*b*) placed forty-two *L. sericata* egg batches in the fleeces of nine sheep under a variety of weather conditions. In sixteen batches more than 50% of the eggs completed development, but in only four of them did larvae hatch out. In the present investigations fleece humidities were determined at 2-day intervals. On only four occasions were humidities suitable for hatching recorded. Conditions necessary for hatching and larval development may occur without sheep becoming attractive to flies as Davies (1948*b*) has shown. There is, therefore, a considerable chance that eggs laid on sheep will not produce myiasis.

The relative absence of attractive sheep and the possible unsuitability of the microclimate are not the only factors which may limit the occurrence of myiasis in Denmark. Fly density and activity are obviously important. Whether host density, as distinct from the density of susceptible sheep, affects the amount of oviposition is not known.

The fact that Danish *L. sericata* did react to attractive sheep is of special significance. It indicates that the absence of myiasis in Denmark cannot be attributed to the occurrence in that country of a physiological race of *L. sericata* differing in oviposition habits from the British species. This conclusion has a bearing on the hypotheses which have been advanced to explain the origin of the phenomenon of myiasis.

Bos (1887*a*) and Froggatt (1904, 1915) considered that myiasis developed as a change in the habit of carrion flies. According to Froggatt, in Australia the myiasis-producing habit originated at a time of severe drought when carrion flies came to live on sheep carcasses and so developed the habit of ovipositing on sheep wool. Recently Crombie (1944) tested the possibility of new food habits acquired by blowfly larvae being passed on to the adults. He showed that flies which developed from larvae reared on a medium containing menthol, or freshly emerged flies which were exposed to menthol, became habituated to the presence of menthol in their environment. He concluded that Froggatt's hypothesis '. . . although improbable, is possible'. It should be noted that in these experiments the effects of habituation were lost before the completion of the pre-ovipositional period. Further, the experiments recorded food preferences, whereas under natural conditions the responses to sheep of *L. sericata* are confined to gravid or almost gravid females and lead to oviposition, not feeding.

So far, no satisfactory evidence has been found to support Froggatt's views. In fact, Tillyard & Seddon (1933) state that drought, rather than favouring the feeding and breeding of blowflies on carcasses, has the opposite effect, the carcasses being rapidly rendered unsuitable for blowfly activity.

The alternative hypothesis considers that oviposition on carcasses and on living sheep occurs in response to similar stimuli. This conception of the origin of myiasis has been carefully argued by Holdaway (1932). It is supported by the investigations of Mackerras & Mackerras (1944), Cragg & Ramage (1945), Cragg (1950) and Cragg & Thurston (1950) which indicate that the responses of blowflies to living sheep are related to the development on them of conditions associated with putrefactive processes. The Danish results support this hypothesis. For, in spite of the fact that myiasis is practically non-existent in Denmark, it was possible to induce *L. sericata* to oviposit on sheep carrying attractants.

On the basis of the qualitative evidence given in Table 1 it would seem that the incidence of myiasis declines as one moves south-eastwards from the mountainous arc formed by the western areas of the British Isles and south-west Norway. The Danish results make it unlikely that this gradient reflects the occurrence within the

species *L. sericata* of races differing in oviposition habits. Instead, it must represent the effects of environment on the various conditions necessary for myiasis. MacLeod (1947) has pointed out the very complex nature of the blowfly-climate-sheep relationship. Until further data are available too much emphasis should not be laid on one environmental factor. In view, however, of the importance of humidity, it is possible that the myiasis gradient largely reflects the importance of rainfall as a factor determining the development of myiasis.

The writer wishes to record his thanks to the many authorities who provided the information on which Table 1 is based. The results obtained in Denmark owe much to the considerable help given by Dr Thamdrup, Konsulent Nislev, Stud. Mag. Rasmussen and Mr Petersen. Thanks are also due to Prof. Bendixen and Prof. M. Thomsen for their interest in the work and to Dr Van Emden for assistance in identifying the *Lucilia* species. The expenses of the investigation were defrayed by a grant from the Agricultural Research Council.

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(*Received* 18 *July* 1949)

A SUCTION TRAP FOR SMALL AIRBORNE INSECTS WHICH AUTOMATICALLY SEGREGATES THE CATCH INTO SUCCESSIVE HOURLY SAMPLES

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(With Plate 7 and 5 Text-figures)

This paper describes the design and operation of a suction trap which takes in air at a constant rate in winds up to 12 m.p.h. The catch is deposited in a collecting tube into which closely fitting disks fall, one every hour, thus segregating the catch into successive hourly samples. The trap is electrically driven; it operates in all weathers and heavy rain does not disorganize the catch.

Introduction

The suction trap was devised in order to sample small, airborne insects, particularly aphids. It sucks in air at a constant rate in winds up to at least 12 m.p.h., which is about the upper limit for winds of biological significance at or just above crop level in Great Britain. The suction trap thus differs from the wind net and the sticky trap, both of which are held passively into the wind and have a catching power which varies according to the wind force.

A suction trap which samples constant quantities of air is more efficient, with small insects in calm conditions, than the traps which depend on the variable flow of natural wind for much of their catching power. As investigations proceed, however, the matter is found to be far from simple; and a description of the comparative performances of the different types of trap and their more theoretical aspects are reserved for a later paper.

Apart from any increased accuracy in estimating insect density which the trap may possess it has some practical advantages over other types of trap. It is clean to use and will work well in any weather. The smaller insects for which it was designed are in excellent condition for preservation and identification. It is particularly suited for work in very calm air and probably in confined spaces where other traps may not be suitable. It does not depend primarily on any response of the insects for its catching power. Its greatest disadvantage may be the necessity for a supply of electricity.

General design

The trap is composed of an electric fan; a copper gauze cone and collecting tube; and a mechanism for segregating the hourly catch (Pl. 7). In addition, there is a Venner time-switch and a transformer. It is mounted so that its opening faces

upwards and the fan revolves in a horizontal plane; wind blowing across the top, therefore, does not enter directly through the opening. Air is sucked in, together with insects, which are blown down the cone into the collecting tube at the lower, pointed end. This collecting tube is of copper gauze which not only allows rain water to run away but is also essential for the proper working of the segregating mechanism. When it is unnecessary to divide up the catch automatically, the collecting tube may be of glass with a filter-paper placed inside impregnated with pyrethrum or tetrachlorethane.

The mechanism for separating up the catch is composed of a pile of metal disks, supported beneath the fan-motor by a pivoted release mechanism. At each hour the time-switch allows a current to pass through the transformer to a solenoid, which, becoming a magnet, pulls the release across and allows a disk to drop down a central rod which guides it to its resting place in the gauze collecting tube (Pl. 7, fig. 3; Text-fig. 2). After a disk drops, the one above falls into its place on the release, ready to drop after another hour has passed. The time-switch then, after a few seconds, turns off the current. Thus after some hours the gauze collecting tube contains a pile of disks with the catches of each hour in the spaces between. The tube with its disks is then detached from the trap and the catch is sorted out.

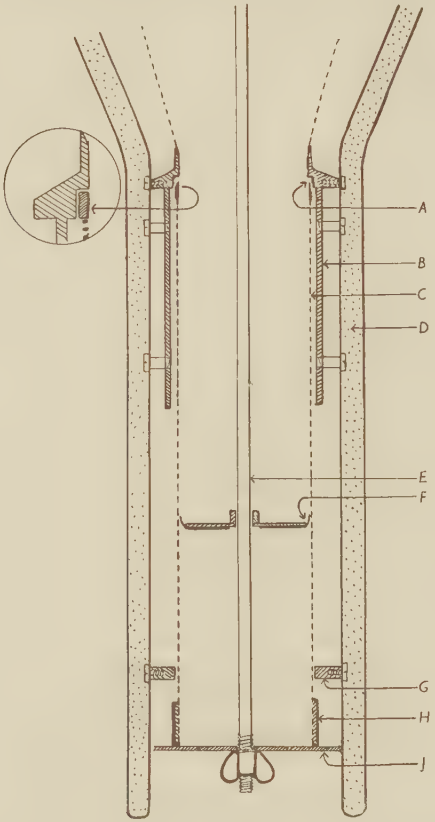
Each disk is fringed with Egyptian cloth which seals off the space between its periphery and the wall of the collecting tube. Before being loaded into the trap the disks are dipped into a petrol ether solution of pyrethrum which evaporates to leave a lethal film giving a rapid knock-down when the insects are blown on to the disk as it lies in the collecting tube; this ensures that the whole catch during the hour, with negligible exceptions, comes to lie on its disk ready to be sealed off by the next one to fall.

The fan unit. This is a Vent-Axia 'Silent Nine' ventilating fan, 9 in. in diameter and connected to a mains supply of electricity. The fan makes a draught of air of approximately 12 m.p.h. through the cylindrical fan casing. The makers supply a grid or a cowl to cover the opening; this is removed for the suction trap so that the fan and opening are exposed.

The unit is designed to be mounted either sideways or upright as in the suction trap, with the motor below the fan. When thus mounted a normal unit, as supplied by the makers, would blow air out through the opening instead of sucking it in. It is necessary, therefore, to state the use to which the unit is to be put, and when ordering to ask for an impellor, which is a fan with reversed blades which sucks the air *in* through the top opening. The unit must *not* be hung upside down with the fan below the motor for this causes the spindle to fall away from its bearing and results in undue wear. When removing a fan care must be taken with the central screw which has a left-hand thread.

One of the radial struts which supports the motor in the centre of the casing is deeply grooved to carry the electric leads from the motor to the plug on the casing. When mounted as a suction trap without the cowl rain will collect in this groove and

run down into the motor. It is essential, therefore, to fill it up with paraffin wax and to bind it over with insulating tape which is finally painted with a waterproof varnish. To avoid rain water shorting the mains plug on the casing this plug is dispensed with and the leads taken directly to the time switch and transformer (Pl. 7).



Text-fig. 1. Section of lower end of cone, collar, collecting tube and iron supports. *A*, internal flange in brass collar into which upper rim of collecting tube fits, with its internal surface flush with that of the collar; *B*, brass collar; *C*, wall of gauze collecting tube; *D*, grooved iron support; *E*, guide-rod; *F*, fringe of Egyptian cloth on disk; *G*, brass ring for centring collecting tube; *H*, lower rim of collecting tube; *J*, centring plate.

These precautions are made necessary by the abnormally exposed position in which the unit, without its protective cowl, is run. They are in no way due to its design as a ventilating fan where the connexions are either indoors or otherwise under cover, and the air flow is in the opposite direction.

The gauze cone and collecting tube. The cone is of copper gauze, 26 mesh to the inch

and 21 in. long. The margin of the wide end is strengthened by a rim of $\frac{1}{8}$ in. brass $\frac{3}{4}$ in. wide which is soldered on the inside of the cone; over this soldered join, a band of thin brass of the same width is clipped. The inner surface of the brass rim fits exactly over the lower metal rim of the fan casing and is securely fastened to it by screws at three places round the circumference. Attached to the cone at the rim, opposite the grooved radial support, is a small brass plate with a rubber-edged hole through which pass the electric leads from the motor. The lower, narrow end of the cone is soldered to the inner-surface of a collar of $\frac{1}{16}$ in. brass tubing, $1\frac{5}{8}$ in. internal diameter, in such a way as to leave a smooth surface on which no insects can lodge; the collar has an internal flange into which the upper end of the collecting tube fits snugly (Text-fig. 1).

An essential feature of the cone is its rigidity, for on this depends the accurate centring of the guide-rod and the successful passage of the disks into the collecting tube. This rigidity is secured by three supports of channelled iron which are bolted to the upper rim and lower collar of the cone; these supports extend beyond the latter in a parallel fashion and, near their distal ends, are screwed with their inner surfaces to a ring of $\frac{1}{8}$ in. brass (Pl. 7, Text-fig. 1).

The collecting tube is of 26-mesh copper gauze, $6\frac{1}{2}$ in. long, $1\frac{3}{8}$ in. internal diameter; it is made up on a cylindrical wooden former. It is kept rigid longitudinally by the soldered join and by two thin strips of brass which are soldered along its length on the outer surface. The lower rim of the tube is a ring of $\frac{1}{16}$ in. brass and the upper rim a very much thinner ring built up of solder and filed smooth; both the upper and the lower rings are on the outside of the tube, leaving the inner surface smooth from end to end.

A gauze tube has several advantages over a glass or metal one: when a disk drops into a tube with solid walls the air is compressed and a small eddy is produced round the edge of the disk as it slides down the tube. This causes small insects to be blown up on to the upper surface of the falling disk instead of remaining below it. With a gauze tube this does not occur, for the disk presses air out at the sides of the tube on its way down. A tube with solid walls provides a pocket of calm air from which insects tend to crawl and to collect at the lower end of the gauze cone. With a gauze tube this does not happen to the same extent and the insects which are rapidly immobilized by the pyrethrum, remain on the upper surface of the topmost disk. The few which crawl back up the tube soon fall down again, or are swept off the sides of the tube by the next disk to fall. An altogether negligible proportion succeed in getting back into the cone. A solid-walled tube, moreover, becomes smeared with a very thin film of pyrethrum extract and small insects soon become stuck to it. This does not occur when the tube is of gauze. A gauze tube allows rain-water to percolate through the sides. Even after very heavy rain no water collects in the tube and the catch is never disturbed by insects becoming washed out from between the disks.

Centring the collecting tube. The disks pass through the collar and down the tube

with a clearance of only $\frac{1}{16}$ in.; this space is, moreover, filled up by the cloth fringe around the edge of each disk. It is essential that these disks should pass unerringly through the collar of the cone and smoothly into the collecting tube. The importance of the rigidity of the cone cannot, therefore, be over-emphasized, for lack of rigidity will lead to inaccurate centring of the guide-rod in relation to the collar and tube, and the disks will either stick in the tube or pile up at the collar and block it.

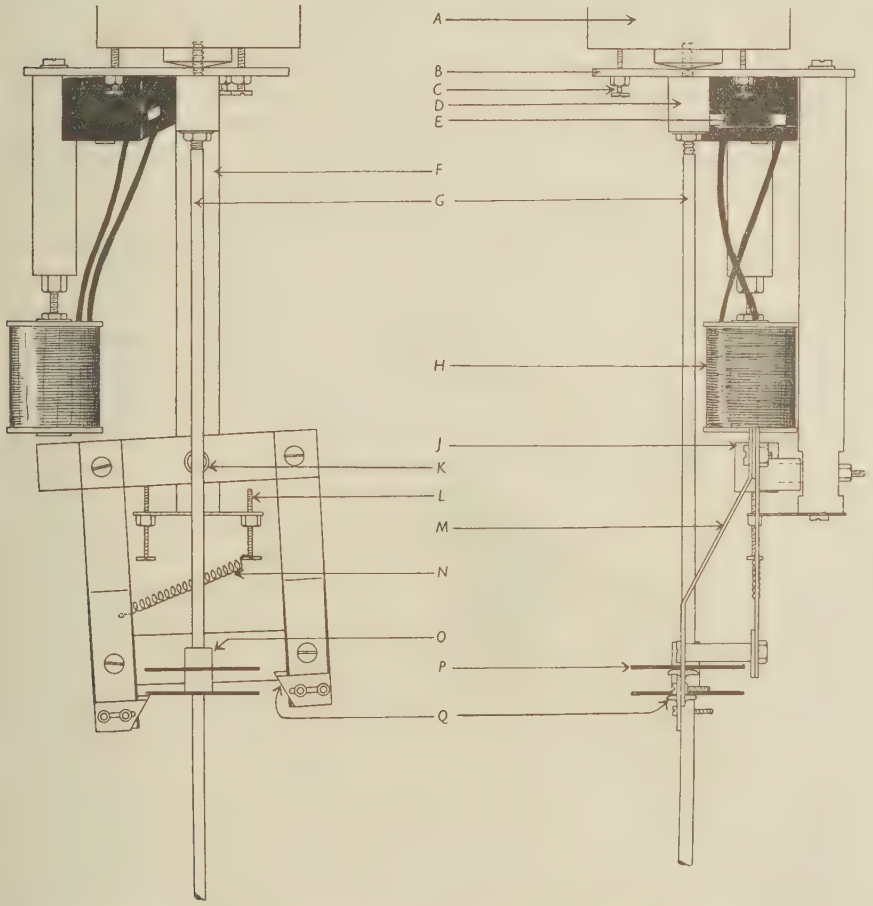
The upper rim of the collecting tube fits snugly into the internal flange of the lower collar of the cone (Text-fig. 1). The soldered join and longitudinal strengthening strips slide through grooves cut in the lowest ring between the three parallel iron supports, and by fitting into them closely ensures that the tube has no lateral play. When the collecting tube is in position it is further secured and centred by means of a centring plate; this is a disk of brass or aluminium which fits exactly between the ends of the parallel iron supports as they project beyond the end of the collecting tube. The centring plate has a central hole through which the threaded end of the guide-rod passes; this thread takes a small wing-nut which tightens the centring disk against the lower rim of the collecting tube and not only closes it at the bottom and keeps it firmly fixed, but also keeps the guide-rod central as it passes down the tube. A few minute holes in centring plate allow rain water to run out.

Centring the guide-rod. The guide-rod, which is of $\frac{3}{16}$ in. tool steel, 24 in. long and threaded for $\frac{3}{4}$ in. at its distal end, is centred primarily by an adjustment at its mounting and is subsequently held in this position by the centring plate at the bottom of the collecting tube. The guide-rod is mounted on a basal plate of $\frac{1}{8}$ in. brass which is bolted firmly into the centre of the motor casing, the maker's name-plate being removed for the purpose. Three set-screws pass through the basal plate and abut on to the motor casing (Text-fig. 2); these enable the basal plate to be tilted one way or another and the guide-rod to be centred with reference to the lower collar of the cone.

The solenoid and disk-release. These are suspended from the basal plate beneath the fan motor and somewhat sheltered from the down-draught of the fan. The arrangement is illustrated in Pl. 7 and Text-fig. 2. Two stout brass rods, 5 in. and $2\frac{1}{2}$ in. respectively, hang from the basal plate. The shorter one carries the solenoid which is $1\frac{1}{2}$ in. long and consists of a large number of turns of 24-gauge enamelled copper wire wound round an insulated iron core, the lower end of which projects beyond the bobbin for a fraction of an inch. This solenoid is, in the present model, overloaded and should be more robust. On the end of the 5 in. rod and at right angles to it a thin brass plate is screwed; just above this the rod is flattened and bored to take a short spindle. On this spindle is pivoted the disk-release which consists of a square frame of $\frac{1}{16}$ in. brass; the spindle passes through the middle of the top side of this frame. A small block of soft iron on the top corner of the frame lies just beneath the end of the solenoid. The square frame does not lie in the same plane as the guide-rod but about 1 in. away; but two brass strips bolted one on each side of the frame are bent so as to lie almost in the same plane as the guide-rod (Text-fig. 2). On the end of each

of these two bent strips a small shelf is screwed so that one is slightly less than $\frac{1}{4}$ in. higher than the other. The lower of these two shelves supports the pile of disks.

When the solenoid is activated the soft iron block is attracted towards it, the frame tilts on its pivot and the lower shelf is pulled from beneath the bottom disk allowing



Text-fig. 2. Front and side view of disk-release mechanism. *A*, casing of fan motor; *B*, basal plate; *C*, set-screws for adjusting plane of basal plate; *D*, seating for guide-rod; *E*, connexion box; *F*, 5 in. brass rod; *G*, guide-rod; *H*, solenoid; *J*, soft iron block; *K*, pivot of release frame; *L*, screws for adjusting degree of movement of release frame; *M*, bent brass bars carrying disk shelves; *N*, spring for returning frame to original position; *O*, collar of disk; *P*, disk; *Q*, shelves for supporting disks.

it to fall down the guide-rod into the collecting tube. At the same time, the upper shelf moves across under the next lowest disk, and catching it, prevents the rest of the disks from sliding down the guide-rod. After a few seconds the time-switch

turns off the current and a coil-spring pulls the frame back into its original position, and the pile of disks falls on to the lower shelf ready for the next disk to be dropped after another hour has passed. It is essential to have a spring of sufficient strength to pull the release back quickly yet not to impede the action due to the solenoid; this is a matter of trial and error. The adjustment of the frame to give the correct amount of movement is made by two small set-screws beneath the upper side. It is advisable that the shelves shall have slightly bevelled surfaces, and that they should be tilted downwards slightly so that the minimum amount of surface is in contact with the lower face of the disk and an undue degree of friction is avoided. The angles of the shelves themselves can be adjusted by screws which fasten them to the frame.

The disks. For a run of 24 hr. twenty-five disks are stacked above the release. Each disk, of thin brass, is $1\frac{1}{4}$ in. in diameter with a central collar $\frac{1}{4}$ in. high and $\frac{5}{16}$ in. diameter through which there is a hole slightly larger than $\frac{3}{16}$ in. in diameter. The collar keeps the disk surfaces a suitable distance apart so that the release shall work correctly and so that the space between the disks shall be sufficient to accommodate a good quantity of small insects. Each disk was made originally to weigh 5 g.; this was found to be far too light to overcome the resistance of the cloth fringe on the sides of the tube, and later a brass washer was soldered to the upper side of each disk increasing the weight to 12 g. which is even then only just heavy enough. As the disks diminish in number above the release the weight of the last two or three tends to be insufficient to give the necessary impetus to the lowest disk, and it is advisable to place a small circular lead weight above the topmost disk to ensure that the last two or three disks fall. On the lower surface of each disk a piece of thin Egyptian cloth is stuck so that it projects as a fringe about $\frac{1}{16}$ to $\frac{1}{8}$ in. wide beyond the edge of the disk; this confines insects to their appropriate place between the disks and prevents them from crawling out or from being washed out by the rain. The cloth is stuck on to the lower surface of the disks with shellac varnish; this withstands fairly rough handling. The disks may then be used for several weeks without the cloth becoming detached. Shellac varnish also dries with a hard surface so that it does not stick to the shelves of the release, neither is it softened nor desolved by the petrol ether and oil with which the disks come into contact.

The time-switch and transformer. The Venner time-switch Type MD 1 SL has a dial marked in minutes with two adjustable pins which may be set at any interval within the hour. One pin switches the current on and the other switches it off. For the suction trap these two pins should be set as closely together as possible so that the solenoid is activated for as short a time as the switch will permit; this is about 30 sec. The switch is placed in parallel with the fan and connects to the transformer and thence to the solenoid.

The transformer brings the mains supply to 15 V. and 4 amp. and ensures a sufficiently powerful kick to pull the lower shelf from beneath the pile of disks.

Care and attention. The care and operation of the disk suction trap are best described by describing the daily routine.

To remove the catch of the previous 24 hr. the centring plate is held firmly against the bottom of the collecting tube while the wing-nut holding it in place is unscrewed. The tube with all its disks is then gently withdrawn from the trap, care being taken not to disturb the disks as they slide from the guide-rod. When the tube and disks are detached a short length of rod is threaded through the disks as they lie piled in the tube and the tube is closed at the top by a spare centring plate; the bottom is already so closed. In the laboratory the tube is placed upright on the bench and the lowest disk dropped out while the one above is held back by the points of forceps; this is repeated till all the disks have slipped down the central rod and lie in a series on the bench. The catch, with negligible exceptions, will lie on the disk surfaces; the insects will be dead or feebly moving and lightly stuck to the disks. Very few are ever found adhering to the walls of the collecting tube. A very small proportion of the catch may be squashed between the collar and the disk above; it has usually been possible to identify aphids damaged in this way.

After the catch has been removed, the disks are brushed free of debris, cleaned by brushing with petrol ether and then dipped into the pyrethrum solution and allowed to dry. An oily rag is pulled through the hole of each disk, and these are threaded on to a short length of rod; they are now ready to be replaced in the trap. The solution of pyrethrum in petrol ether (two or three drops of 25% pyrethrins in 20 c.c. petrol ether) should be prepared in advance and allowed to stand for a day. This ensures that the resinous material separates out in the stock bottle and not on the disks where it will cause them to stick together and often to fail to drop from the release.

When the full tube with its disks is removed from the trap, a fresh set is inserted for the next 24 hr. Before this is done, however, the guide-rod is cleaned and oiled as follows. A brass ferrule lined with copper gauze and attached to a length of flexible brass wire is filled with light oil and pushed once or twice up and down the guide-rod; this prevents rust from accumulating and allows the disks to fall easily. The end of the rod on which the fresh set of disks are carried is then placed end to end with the guide-rod and all twenty-five disks are slipped up on to it in one operation. They are pushed up into the trap and past the release with the oiling ferrule; to facilitate this the shelves on which the disks rest are cut at an angle on their undersides so that the disks will slide past them (Text-fig. 2). The collecting tube and centring disk are then replaced, and the wing-nut on the end of the guide-rod screwed up. A disk is released by a press-button on the time-switch; it falls into the tube and makes a lethal surface for the first hour's catch. Twenty-four disks remain in place, and the trap is ready for the next run.

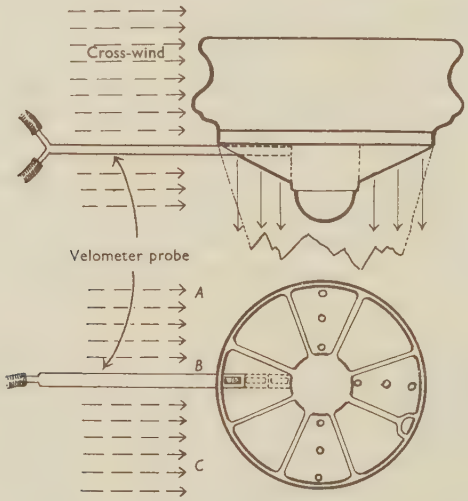
It is necessary to oil the pivot of the release occasionally to avoid stiffness, but care must be taken not to use too much oil for an excess will be blown from the release on to the gauze cone causing insects to stick there instead of going into the collecting tube. To prevent insects from becoming deposited on the oily surface of the release frame or on to the disks above the release, a cylindrical cellophane guard

may be attached to struts screwed to the basal plate so that it fits up against the motor casing and extends downwards for about 8 in., thus enclosing most of the release mechanism.

The whole apparatus, when maintained with care, works efficiently and, once a routine is established, will continue to do so for periods of several weeks without trouble.

The effect of cross-winds on volume of air sampled

The volume of air sampled by the fan unit was estimated by measuring the air-speed within the casing at twelve different places while the unit was operating in calm air. This air-speed was measured with a Metrovic velometer; Text-fig. 3 indicates the twelve positions. The minimum area of free space in a cross-section



Text-fig. 3. The position of the velometer probe while measuring the air-speed through the fan unit. In the lower figure the twelve positions are indicated by small circles; *A*, *B* and *C* are the positions at which the speed of the cross-wind was measured.

of the unit was measured, and allowing for the slight differences in air-speed in the inner, middle and outer zones of the fan casing, the volume of air displaced in 1 hr. was calculated as 19064 cu.ft./hr.

The gauze cone was then attached to the unit and the air-speed at the twelve positions again measured. The mean air-speed through the unit, with and without the cone is shown in Table 1, where it can be seen that the air-speed, and therefore the volume of air displaced by the unit, is not appreciably diminished by the presence of the cone.

Measuring the effect of cross-winds on the rate of air intake. In the original model it was found necessary to make the correction for the effect of cross-wind on the

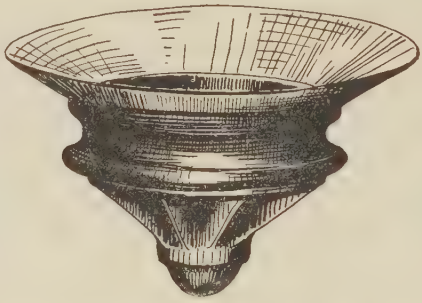
amount of air sampled by the trap; and since many field experiments have been made in this way (and will be described in a later paper) the procedure for estimating and for making the correction is given.

TABLE 1. *The effect of cross-winds, 21 in. gauze cone and 3 in. flared rim on the air intake of the 9 in. Vent-Axia fan unit*

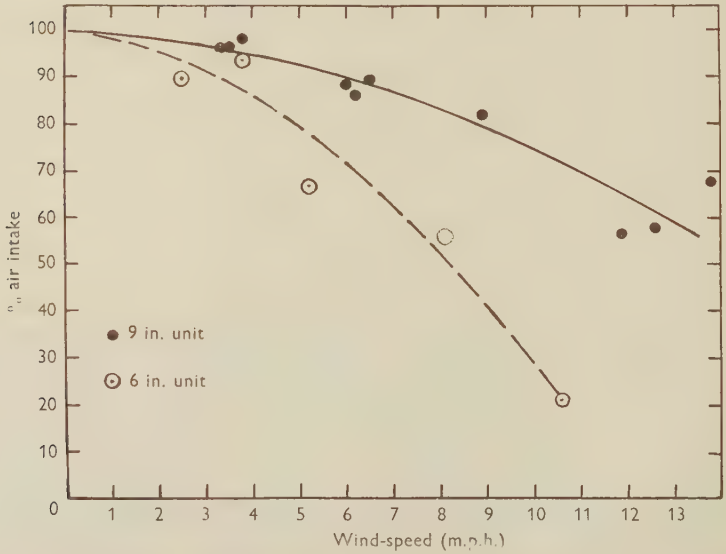
Mean cross-wind speed (m.p.h.)	Mean wind-speeds through the fan unit (m.p.h.)			
	Without flared rim			With flared rim, with cone
	No cone	With cone		
0	—	12·2	11·4	11·5
3·3	—	11·7	—	—
3·8	—	—	11·2	11·7
0	11·4	11·6	11·6	11·7
6·0	—	10·3	—	—
6·2	—	—	10·0	11·5
11·3	—	8·2	—	—
19·0	—	3·6	—	—
0	12·2	12·0	11·6	11·9
8·9	—	9·8	—	—
11·9	—	—	6·6	11·5
13·8	—	8·1	—	—
0	—	11·8	—	—
3·5	—	11·3	—	—
6·5	—	10·5	—	—
12·6	—	6·8	—	—

A large aerofoil fan operating in a horizontal duct 18 in. in diameter was used to blow air, from which the turbulence was not eliminated, past and across the top of the suction trap; the speed of this cross-wind was measured with the velometer at positions *A*, *B* and *C* (Text-fig. 3) $\frac{1}{2}$ in. above the rim of the trap (the latter not working while this measurement was taken). The trap fan was then switched on and the air-speed in the fan-casing of the trap was measured once more in the same twelve positions as before, this time of course with the cross-wind blowing. The percentage reduction in mean wind-speed, within the trap with the cross-wind as compared to the value in still air, was taken as the percentage reduction in the quantity of air was sampled. This procedure was repeated with winds of various speeds blowing across the trap; the speed of the cross-wind was varied by a pulley system which enabled the relative positions of the trap and cross-wind fan to remain constant at a distance of about 8 ft. In these tests the Vent-Axia fan and the gauze cone below were placed in the cross-wind which blew past the whole trap, not merely over the top of it. The air-speeds are shown in Table 1 and the smoothed curve for percentage reduction in Text-fig. 5. If the run of the wind during the trapping period in the field is measured, a correction may thus be applied for the effect of wind on the trap, by means of the graph in Text-fig. 5.

Vent-Axia fans are made for two mains voltage ranges, i.e. 200/220 and 230/240 V.; it is necessary to stipulate the exact voltage and current supply when ordering.



Text-fig. 4. 9 in. Vent-Axia unit with flared rim: not strictly to scale.



Text-fig. 5. Curves showing percentage reduction in rate of air intake by 6 and 9 in. Vent-Axia units in cross-winds of various speeds. Data in Tables 1 and 2; curves fitted by eye.

A slight drop in voltage from 240 down to 210 V. occurs at Rothamsted but this does not diminish the air-speed of the fans.

Elimination of effects of cross-winds on rate of air intake. Since the experiments described above were made, it has been found that if the trap is fitted with a flared rim 3 in. wide and with an included angle of 30° (Text-fig. 4), the effect of wind on the air intake of the trap is entirely eliminated up to at least 12 m.p.h. This should enable direct comparisons to be made between catches without the need for the correction

Fig. 1

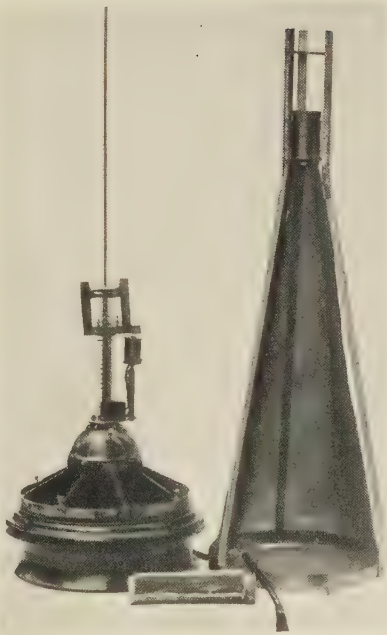


Fig. 2

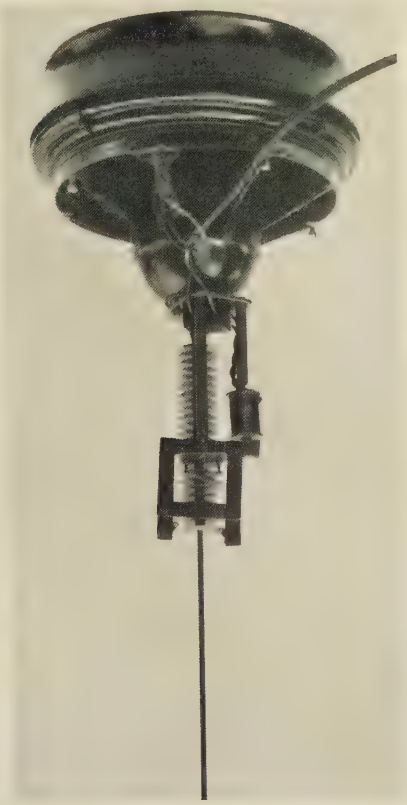


Fig. 3

factor to be applied. Table 1 shows a series of measurements of air-speed through the fan unit when the trap with and without the flared rim is subjected to cross-winds of various speeds.

Other types of fan units

A smaller suction trap, without the segregating mechanism has been in use; this is made up from a Vent-Axia 'Silent Six' which is a smaller unit 6 in. in diameter. According to calculations made in a similar fashion to those described above for the 9 in. trap, this unit displaces 5475 cu.ft. of air/hr. A gauze cone 14 in. long does not diminish this rate greatly. Details of its performance with and without a flared rim are given in Table 2 and Text-fig. 5.

Table 2. *The effect of cross-winds, 14 in. gauze cone and 3 in. flared rim on air intake of 6 in. Vent-Axia fan unit*

Mean cross-wind speed (m.p.h.)	Mean air-speeds through the fan casing (m.p.h.)		
	Without flared rim		With flared rim, with cone
	No cone	With cone	
0	6·7	6·4	—
0	6·6	6·4	—
0	—	6·8	7·1
3·8	—	6·4	—
6·1	—	—	6·9
8·1	—	3·8	—
10·7	—	1·4	—
0	—	5·9	7·1
2·5	—	5·5	—
5·3	—	4·1	—
7·7	—	—	6·6
0	—	—	6·9
11·0	—	—	6·3

Vent-Axia Ltd. supply units to run from 12 V. storage batteries. Although I have had no experience of these they may under some circumstances be useful for field work where there are no other sources of electrical power. A 6 in. unit run off a 12 V. battery takes 1·3 amp. and a 9 in. unit, 2·8 amp.: a battery with 20 amp.-hr. would, therefore, work a 6 in. unit for 15 hr. and a 9 in. unit for 7 hr. It is, of course, possible to get batteries which would run the traps for longer than this.

My best thanks are due to Mr I. Long who made the original model of the disk trap, to Mr L. R. Taylor for operational improvements and to Mr R. Marriott of Vent-Axia Ltd. for many kindnesses.

EXPLANATION OF PLATE 7

Fig. 1. External appearance; trap in correct position but without flared rim.
Fig. 2. Gauze cone, collecting tube and fan unit with disk segregating mechanism.
Fig. 3. Fan unit with disk segregating mechanism.

(*Received* 20 *May* 1949)

THE INSECTICIDAL ACTIVITY OF SOME SYNTHETIC ORGANO-PHOSPHORUS COMPOUNDS

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The toxicity of a series of organo-phosphorus compounds to the overwintering eggs of *Aphis pomi* Deg., *Operophtera brumata* (L.) and *Metatetranychus ulmi* (Koch) was examined by a laboratory technique. The eggs of *M. ulmi* were highly resistant to all the compounds tested, those of *Operophtera brumata* were most susceptible to *p*-nitrophenyl diethyl thionphosphate (E 605) and those of *Aphis pomi* to *p*-nitrophenyl diethyl phosphate (E 600). With analogues of the last-mentioned compound there is evidence that their toxicity to *A. pomi* eggs is associated with the presence of electron-attracting substituents in the benzene nucleus. A number of organophosphorus compounds, including hexaethyl tetraphosphate and *p*-nitrophenyl diethyl thionphosphate, were highly toxic under laboratory conditions to the active summer stages of *Metatetranychus ulmi*, but the summer eggs of this mite were rather more resistant. Field trials of hexaethyl tetraphosphate and *p*-nitrophenyl diethyl thionphosphate for the control of the aphids *Brachycaudus helichrysi* (Kalt.), *Phorodon humuli* (Schr.) and *Aphis pomi* Deg. showed these substances to be effective substitutes for nicotine.

One of the major results of recent research in plant protective chemistry has been the development, by Schrader and his collaborators in the laboratories of the I.G. Farbenindustrie, of a new class of synthetic organic insecticides, the organophosphorus compounds. The original German work was carried out just prior to and during the war of 1939–45 and preliminary information on the topic only became available to us late in 1945 (personal communications from Drs H. Shaw and W. S. Rogers) when the researches described in this paper were initiated. Subsequently, more detailed accounts were obtained by American and British workers who interrogated Schrader (Thurston, 1946; Martin & Shaw, 1947) and a memorandum on the subject has been prepared by Schrader (1947). The organophosphorus insecticides include not only those acting as contact and/or stomach poisons, but also a highly interesting series—the so-called 'systemic insecticides' of Martin & Shaw (1947) and Bennett (1949)—which are absorbed and translocated by plants which thereby become insecticidal. We have not, however, investigated the latter type of compound, and the present account is devoted entirely to substances of the kind shown by Schrader and other workers (e.g. Ludvik & Decker, 1947; Bennett & Martin, 1948) to possess contact insecticidal properties.

In 1939, Schrader and his colleagues demonstrated the toxicity to aphids and other insects of a product obtained by the reaction of phosphoryl chloride with

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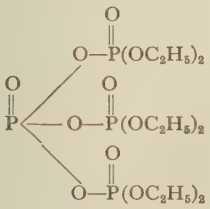
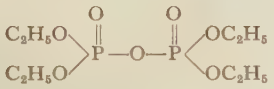
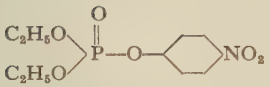
triethyl phosphate. This substance, known commercially as 'Bladan' ('HETP') was considered by Schrader to be hexaethyl tetraphosphate (I), although later work, reported by Coates (1949), has shown that it is probably a mixture of linear polyphosphoric esters up to and including hexaethyl tetraphosphate. Somewhat similar, though greater, toxic properties were also ascribed to tetraethyl pyrophosphate ('TEPP') (II), synthesized earlier by Nylen (1933) and generally considered to be the active principle of 'Bladan'. Broadly speaking, these compounds appeared to resemble nicotine in their high toxicity to aphids and other small soft-bodied insects such as young lepidopterous larvae, but they were found to be readily hydrolysed in water or alkaline solutions to yield non-toxic products. The desire to obtain a compound which would remain stable in alkaline spray-formulations and which would prove effective in the control of the Colorado beetle, *Leptinotarsa decemlineata* (Say), eventually resulted in the development of the highly toxic and stable substances, *p*-nitrophenyl diethyl phosphate ('E 600', III) and the corresponding thionphosphate ('E 605', parathion, IV). Information is also available now on the toxicity of a number of analogues of these substances, mainly to aphids (Schrader, 1947).



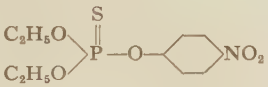
I



II



III



IV

Since most of the reported early work on these compounds consisted of laboratory-scale tests with aphids, it seemed desirable to us to concentrate on three aspects which had not then been studied to the same extent. These were: (*a*) the toxicity of organo-phosphorus compounds to the overwintering eggs of a number of fruit pests; (*b*) their possible value in controlling the summer stages of the fruit tree red spider mite, *Metatetranychus ulmi* (Koch)—a pest whose resistance to the commonly used insecticides makes its control difficult; and (*c*) their value, relative to nicotine, for the field control of aphids. Further, it was desired to examine as long a series as possible of other organo-phosphorus compounds in the hope of elucidating some relations between toxicity and molecular constitution and possibly of finding compounds of even greater toxicity than those previously described.

Laboratory tests

Methods and materials. The overwintering eggs of the green apple aphid, *Aphis pomi* Deg., the winter moth, *Operophtera brumata* (L.), and the red spider mite, *Metatetranychus ulmi* (Koch) were employed in these tests. The methods used in dealing with the first two species have been described in detail by Steer (1938) and Tuft (1944). In the case of the mite, supplies of overwintering eggs were obtained by allowing an infestation to develop in the greenhouse on potted Worcester Pearmain apple seedlings during the late summer (Massee, 1947). Although, under field conditions, overwintering eggs are laid mainly on the second year or older wood, no difficulty was experienced in obtaining large numbers of eggs on the young seedlings—in fact, towards the base of the shoot, the eggs were clustered together many layers thick, and this part of the stem was always discarded as it was thought that penetration of the test liquids to the lower layers might prove unsatisfactory. Tests were then made by selecting egg-bearing shoots in the spring, treating by immersion for 10 sec. and allowing to drain and dry by inserting the lower ends of the shoots in trays of moist sand. Towards the time when hatching of normal eggs was to be expected, short lengths of treated shoot were each placed in small glass tubes tightly closed by fine-mesh cambric, and the mites allowed to hatch into these tubes where they subsequently died. After hatching was complete in the control series, the number of mites in each tube was recorded and the number of unhatched eggs still attached to the shoot was counted after removing them by treatment for 3 min. at 80° C. with alcoholic caustic potash and filtering them through a Buchner funnel using a filter-paper marked into squares of a convenient size to facilitate counting under a low-power binocular microscope. All tests with overwintering eggs were made in quintuplicate and the number of eggs used for testing each concentration on any one occasion varied from about 100 for *Operophtera brumata* to over 300 for *Aphis pomi.*

Previous workers do not appear to have described methods for the large-scale quantitative testing of insecticides against the summer eggs of *Metatetranychus ulmi*, but the following method, although tedious, was satisfactory and was used throughout this work. Infested apple leaves were collected in the field and those bearing about 100 eggs per leaf were selected for tests. All active stages of the mite were then removed from the leaf by suction through a fine glass tube and the remaining eggs on the lower surface of the leaves were counted beneath a squared glass plate under a binocular microscope. Leaves were then dipped for 10 sec. in the washes being examined and, after draining and drying, the petioles were lightly coated with petroleum jelly and each leaf placed with its petiole dipping into water in a small glass tube. The leaves and tubes were then transferred to a moist chamber where they remained until hatching was complete (10–12 days under laboratory conditions). The unhatched eggs were then counted as before. Maintenance of moist conditions was found essential to prevent desiccation of the leaves, and coating

of the petiole with petroleum jelly was necessary to prevent capillary creep of water on to the leaf-surface—a phenomenon which occurred readily when the dipping solution contained a surface-active agent.

Ideally it would have been desirable to examine the susceptibility of all the summer development stages of the mite to the potential acaricides, but their small size and activity made this difficult and all the tests described below were made with adult females which, being largest and rather less active, were easiest to count. Leaves bearing twenty to fifty adult females were dipped in the test-solutions, allowed to drain and dry, the petioles vaselined and then placed in moist chambers as before for 24 or 48 hr., after which counts of dead and living females were made under a microscope, the other stages being ignored. Tests showed that mites were repelled by the vaseline barrier at the base of the leaf, only a very small proportion being trapped in it, and that dead mites were retained on the leaves—usually in the hairs on the lower surface—and were not lost during the course of an experiment. Tests were again made in quintuplicate.

A criticism of the techniques used is that, in order to maintain the leaves in a satisfactory state, they had to be placed in an atmosphere almost saturated with water-vapour, and that the toxicity of substances tested under these conditions might differ from that at lower humidities such as prevail in the field. This difficulty seems impossible to overcome in such tests, as the necessary counts can only be made on leaves removed from the tree, and these invariably wilt if not kept in a moist environment (cf. Jary & Austin, 1938, who used a similar method in dealing with the eggs of the related mite, *Tetranychus telarius* L.). A second criticism might be that the closed moist-chambers permitted the development of a fumigant effect from substances of high vapour pressure. There was no evidence of such an effect in our experiments, this point having been checked with untreated control leaves in the same chamber, but where it is suspected in similar work of this kind, the accumulation of toxic vapour concentrations could probably be prevented by arranging for a slow current of moist air to be drawn through the chambers.

Since some of the commercially available organo-phosphorus insecticides are mixtures of complex composition details are given of the nature of the materials used. The term 'hexaethyl tetraphosphate' is used to denote the reaction product obtained in the normal manner by heating phosphoryl chloride (1 mol.) with triethyl phosphate (3 mol.) at a temperature of 140° C. (Schrader, 1947). In those cases where the proprietary formulations of *p*-nitrophenyl diethyl phosphate and the corresponding thionphosphate are specifically mentioned in the text and tables, these were German samples in which an emulsifier of the 'Igepal' type had been incorporated during manufacture. In other cases, the *p*-nitrophenyl diethyl thionphosphate was a commercially pure sample containing 75% of the active material, the remaining 25% being impurities introduced during manufacture. These impurities consisted mainly of triethyl phosphate, di(*p*-nitrophenyl) ethyl thionphosphate and some free *p*-nitrophenol. All other organo-phosphorus compounds

used were authentic specimens with boiling-point or melting-point in close agreement with those recorded in the literature, and in the few instances where new compounds have been tested, their chemical identity had previously been established by Mr T. V. Healy of the Ministry of Supply, Chemical Defence Experimental Station, Porton. The melting- or boiling-points of the compounds tested are quoted in Table 2. Except for the proprietary compounds mentioned above, all the substances tested were formulated in the same way. Concentrates were prepared containing 10% w/v of the organo-phosphorus compound and 2·5% w/v of the emulsifier Triton X–100 (a polyethylene glycol mono-*iso*-alkyl aryl ether) in ethyl alcohol. Each concentrate was then diluted fifty times with distilled water to give test-washes containing 0·2% w/v of the phosphorus compound, 0·05% w/v emulsifier and 2·0% v/v ethyl alcohol. A second series of test-washes containing 0·05% of the toxicant was prepared by dilution of the concentrate with 2·0% v/v ethyl alcohol and 0·05% w/v Triton X–100 in distilled water. In all tables the concentrations are expressed in terms of the pure (or commercially pure) substance.

(*a*) *Ovicidal activity of organo-phosphorus compounds*

(i) The early experiments on overwintering eggs were carried out in 1946 and 1947 and were confined to the examination of variants of the three substances which were then in the process of commercial development, i.e. hexaethyl tetraphosphate, *p*-nitrophenyl diethyl phosphate and the corresponding thionphosphate. A selection of the results obtained is given in Table 1, where each figure for percentage hatch is followed by its standard error.

Table 1. *Percentage hatch of overwintering eggs treated with organo-phosphorus compounds*

Compound	Conc. %	Species		
		Aphis pomi	*Operophtera brumata*	*Metatetranychus ulmi*
Hexaethyl tetraphosphate	0·4	9·3 ± 1·9	—	93·9 ± 3·72
	0·2	14·3 ± 5·3	—	90·5 ± 4·74
	0·1	44·9 ± 2·4	—	99·7 ± 0·32
	0·05	60·0 ± 5·0	—	95·3 ± 1·20
p-Nitrophenyl diethyl phosphate (E 600) (proprietary formulation)	0·2	0·0	—	85·2 ± 3·49
	0·1	0·0	—	92·4 ± 1·99
	0·05	0·1 ± 0·04	—	92·2 ± 1·99
	0·025	0·0	—	96·4 ± 0·56
	0·0125	0·0	71·0 ± 5·81	—
	0·00625	0·1 ± 0·07	84·3 ± 3·81	—
p-Nitrophenyl diethyl thionphosphate (E 605) (proprietary formulation)	0·2	—	—	87·4 ± 5·45
	0·1	—	—	87·0 ± 1·43
	0·05	—	—	87·8 ± 5·03
	0·01	65·2 ± 3·51	8·6 ± 2·21	—
	0·005	57·6 ± 4·54	45·6 ± 8·53	—
	0·0025	75·2 ± 4·52	58·0 ± 8·35	—
Untreated control	—	75·1 ± 5·8	91·8 ± 1·96	96·3 ± 0·66

These and very similar confirmatory results indicate a few interesting points: First, it seems that none of the three compounds is appreciably toxic to the overwintering eggs of *Metatetranychus ulmi*, even when used at the quite uneconomic concentration of 0·2%. Secondly, hexaethyl tetraphosphate has comparatively little effect on the eggs of *Aphis pomi* (and, as subsequent work showed, it is equally ineffective against eggs of the winter moth). Thirdly, it seems that *p*-nitrophenyl diethyl phosphate possesses remarkable toxicity to the eggs of *Aphis pomi* while being much less toxic to those of *Operophtera brumata*. Finally, the thionphosphate gives a preferential kill of the lepidopterous eggs.

(ii) The remaining tests carried out against overwintering eggs were designed to cover a wide range of organo-phosphorus compounds in a semi-quantitative fashion, rather than to obtain precise estimates of relative toxicity. The substances were accordingly tested at 0·2 and 0·05% w/v and were uniformly formulated as described above. A selection of the results, quoted in Table 2, are expressed in terms of percentage mortality after allowing for control deaths (Abbott, 1925). The standard errors of these figures were generally of the order of 5–10% of the mortality figures, and though this did not always ensure statistical homogeneity it is probably as low as can reasonably be expected in insecticide tests of this kind carried out with field-collected material (cf. Bennett, Kearns & Martin, 1947, for a short discussion of homogeneity in data of this kind).

A detailed discussion of these results is postponed to a further section.

(*b*) *Toxicity to summer stages of* Metatetranychus ulmi

A less extensive series of compounds was employed in these tests owing to the shortage of some of the substances. In general, however, an attempt was made to include all those substances which had shown any promise in the ovicide tests described above. The more important results are summarized in Table 3, the percentage mortality data having again been adjusted in the light of control deaths.

(*c*) *Discussion of laboratory test results*

The results show the highly specific nature of the organo-phosphorus insecticides. Hexaethyl tetraphosphate is highly toxic to the adult females of *Metatetranychus ulmi* at a concentration as low as 0·025% but has little effect on its summer eggs or on the overwintering eggs of any of the three species against which it was tested. Again, *p*-nitrophenyl di*iso*propyl phosphate, which is highly toxic to the eggs of *Aphis pomi* and the adult *Metatetranychus ulmi*, is markedly less toxic to the summer eggs of the mite and hardly affects its winter eggs or those of the winter moth. The best all-round performance seems to have been given by *p*-nitrophenyl diethyl phosphate and the corresponding thionphosphate, but the winter eggs of the red spider mite are only slightly susceptible to these compounds and a rather high concentration of either one would seem necessary to achieve simultaneous control of the eggs of *Aphis pomi* and *Operophtera brumata*.

TABLE 2. *Toxicity of some organo-phosphorus compounds to overwintering eggs of fruit pests*

Compound	Percentage mortality					
	Aphis pomi		*Operophtera brumata*		*Metatetranychus ulmi*	
	0·2 %	0·05 %	0·2 %	0·05 %	0·2 %	0·05 %
Triethyl phosphate (b.p. 216° C.)	1·4	0·0	3·1	7·1	19·9	10·9
Triphenyl phosphate (m.p. 49·5° C.)	14·1	0·0	8·0	6·4	7·6	26·8
Tri-*o*-tolyl phosphate (b.p. 405° C.)	0·0	7·1	5·8	7·9	13·3	0·0
Triphenyl phosphine (m.p. 79° C.)	13·9	5·1	3·4	3·6	3·0	0·0
Hexaethyl tetraphosphate (not distillable)	7·4	1·1	6·2	3·7	0·0	7·0
p-Nitrophenyl diethyl thionphosphate (75 % pure)	100·0	78·6	95·4	90·8	39·1	9·6
Diethyl acetylphosphate (b.p. 110° C./6 mm.)	5·7	8·4	8·7	1·3	0·0	12·3
p-Nitrophenyl diethyl phosphate (b.p. 174° C./1 mm.)	99·6	100·0	99·1	—	47·9	13·3
p-Nitrophenyl di*iso*propyl phosphate (not distillable)	99·9	100·0	7·8	4·4	20·7	13·7
Diphenyl ethyl thionphosphate (b.p. 184° C./1 mm.)	0·0	8·1	8·5	4·8	25·0	13·4
p-Nitrophenyl dichlorothionphosphonite (m.p. 104° C.)	40·4	10·3	—	10·2*	20·0	6·6
Diphenyl chlorothionphosphonate (m.p. 64° C.)	25·9	4·9	4·0	3·0	9·2	8·9
Tri-(*p*-nitrophenyl)thionphosphate (m.p. 165° C.)	5·3	0·6	1·9	4·3	12·9	0·0
Tri-(*p*-chlorophenyl)thionphosphate (b.p. 255° C./5 mm.)	15·7	13·0	1·6	1·9	3·6	24·6
Tetraethyl pyrophosphate (b.p. 145° C./1 mm.)	3·3	2·6	7·2	6·5	5·4	0·0
Diethyl 1-carbethoxyprop-1-en-2-yl phosphate (b.p. 140° C./3 mm.)	100·0	86·3	18·3	2·3	15·9	4·6
Phenyl diethyl phosphate (b.p. 136° C./1 mm.)	59·3	2·9	2·5	3·3	11·6	10·9
p-Chlorophenyl diethyl phosphate (b.p. 142° C./2 mm.)	93·9	14·6	2·5	2·2	11·4	5·7
Phenyl diethyl thionphosphate (b.p. 140° C./7 mm.)	39·9	3·7	1·7	1·6	13·3	20·0
Triphenyl thionphosphate (m.p. 48° C.)	19·0	23·7	0·0	3·7	22·5	0·0
Tetraethyl dithionpyrophosphate (b.p. 130° C./2 mm.)	100·0	54·6	24·0	4·6	5·3	0·0
Tetraethyl monothionpyrophosphate (b.p. 133° C./2 mm.)	12·0	2·4	19·8	1·6	0·0	0·0
Pyrophosphoric tetrakisdimethylamide (b.p. 146° C./3 mm.)	9·0	0·0	1·4	0·0	1·4	1·0
Control hatch	69·9		97·1		81·2	

* Tested at 0·1 % concentration.

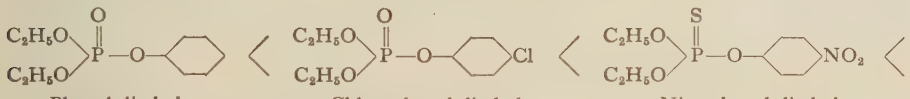
It is doubtful whether any general correlations between toxicity and molecular constitution can be established without a far deeper understanding of the physiological processes involved in insecticidal activity and the relative importance of structural features promoting rapid penetration of cellular and extracellular mem-

branes as against those conferring on the molecule the properties of a cytoplasmic poison. Nevertheless, the partial success which has attended generalizations of more limited scope in this and related fields encourages us to draw attention to a few facts which might lead to further profitable work on the insecticidal properties of these compounds. It is immediately apparent from the data relating to *Aphis pomi* eggs that high toxicity is never associated with unsubstituted alkyl or aryl phosphoric or thionphosphoric acids (e.g. triethyl phosphate, triphenyl phosphate and phenyl

TABLE 3. *Percentage mortality of summer stages of* Metatetranychus ulmi *treated with organo-phosphorus compounds*

	Percentage mortality			
	Summer eggs		Adult females	
Compound	0·1 %	0·025 %	0·1 %	0·025 %
p-Nitrophenyl diethyl thionphosphate	99·5	67·6	95·3	95·3
p-Nitrophenyl diethyl phosphate	85·1	36·5	100·0	98·8
Hexaethyl tetraphosphate	14·1	12·6	99·6	97·9
p-Chlorophenyl diethyl phosphate	3·4	—	—	—
p-Nitrophenyl di*iso*propyl phosphate	80·0	49·4	98·2	98·6
Tetraethyl dithionpyrophosphate	33·0	12·0	90·8	—
Phenyl diethyl phosphate	5·5	0·0	40·0	—
Diethyl 1-carbethoxyprop-1-en-2-yl phosphate	4·8	0·0	100·0	93·9
Phenyl diethyl thionphosphate	0·0	0·0	78·9	—
Tetraethyl pyrophosphate	13·2	12·7	97·9	92·2
Triphenyl phosphine	10·0	3·4	41·7	—

diethyl phosphate are all of low toxicity, the best results being obtained with phenyl diethyl phosphate which gave a kill of 59·3 % at a concentration of 0·2 %). Furthermore, the presence of three similar substituted organic groups (as in tri-(*p*-nitrophenyl)thionphosphate and tri-(*p*-chlorophenyl)thionphosphate) seems likewise inimical to high toxicity. The most effective compounds in this series of substituted phosphoric and thionphosphoric esters all seem to possess two alkyl groups and a single substituted aryl group and may be arranged in order of increasing toxicity as follows:



Phenyl diethyl phosphate — *p*-Chlorophenyl diethyl phosphate — *p*-Nitrophenyl diethyl thionphosphate

p-Nitrophenyl diethyl phosphate = *p*-Nitrophenyl di*iso*propyl phosphate

The steadily increasing toxicity which seems to result from the introduction, first of —Cl and then of —NO_2, into the benzene ring runs parallel to the increasingly electron-attracting character of the substituent. It is therefore suggested that, at any rate as far as the eggs of *Aphis pomi* are concerned, high toxicity is correlated with the presence of two alkyl groups and a strongly electron-attracting substituted aryl group. Data are not sufficiently extensive to confirm this hypothesis for the summer stages of *Metatetranychus ulmi* or the overwintering eggs of *Operophtera brumata*, but the few results obtained with these forms are not inconsistent with it. The other compounds tested do not form related series, but the rather high toxicity of diethyl 1-carbethoxyprop-1-en-2-yl phosphate and tetraethyl dithionpyrophosphate to *Aphis pomi* eggs suggests further types of organo-phosphorus compounds for investigation.

Considering the results from a horticultural standpoint, it is interesting to note first that the adult females of *Metatetranychus ulmi* are readily killed by a number of compounds, whereas the summer eggs are decidedly more resistant. It seems, therefore, that particular attention needs to be paid to the summer eggs if attempts are to be made to develop an organo-phosphorus compound which will give really adequate summer control of this pest by means of a single application of spray. Other laboratory data not quoted in Table 3 suggest that even *p*-nitrophenyl diethyl phosphate and the corresponding thionphosphate at 0·05% concentration might still permit a sufficient hatch of eggs to allow subsequent build-up of the population under favourable conditions, while it is evident that claims for the successful control of tetranychid mites with hexaethyl tetraphosphate and tetraethyl pyrophosphate (e.g. by Fayette, Hensill & Cassil, 1946) depend on a second spray-application to kill the immature mites which hatched from eggs surviving the first treatment. The summer eggs seem to be most susceptible to *p*-nitrophenyl di-alkyl esters of phosphoric and thionphosphoric acids and the suggestion that high toxicity is bound up with the electron-attracting character of the nitro-group in this type of compound might well be followed up with this developmental stage of the mite.

The great resistance shown by the winter eggs of *M. ulmi* to all the compounds here examined makes it unlikely that the organo-phosphorus compounds investigated will ever be of great value as winter-washes, a view tending to be confirmed by the fact that no one of them will give a high kill of both aphid and winter moth eggs at economically practicable concentrations.

Field trials

These were designed mainly to compare hexaethyl tetraphosphate (HETP) and *p*-nitrophenyl diethyl thionphosphate (E 605) with nicotine for the field control of aphids. Three trials were made; in all cases the three materials compared were uniformly formulated with concentrations in the final wash of 0·5% v/v acetone, 0·025% w/v *cyclo*hexylamine dodecyl sulphate (M.B. 320) and 0·015% w/v dioctyl sodium sulphosuccinate (Aerosol O.T.) so that wetting and spreading properties were the same throughout.

Trial 1. *Leaf-curling plum aphid*, Brachycaudus helichrysi (*Kalt.*)

The material available for this trial comprised ninety-four plum trees (variety Czar) severely infested with *B. helichrysi* and interplanted with the variety Victoria, on which only a light infestation had developed and which were excluded from the trial. The trees had received no spray treatments during the previous two seasons and when first seen the infestation had reached an advanced stage. Extensive sample counts showed that over 80% of the leaves were badly curled and that even the uncurled leaves usually bore a few aphids. For various reasons it was necessary to adopt a somewhat primitive experimental layout, the trees being divided into eight plots among which the following four treatments were randomized:

(1) 0·05% hexaethyl tetraphosphate;
(2) 0·05% *p*-nitrophenyl diethyl thionphosphate;
(3) 0·05% nicotine;
(4) unsprayed control.

Spraying was done under satisfactory conditions on 10 May 1948, but migration of the insects to their summer hosts was beginning when spraying started. A detailed qualitative examination of the trees 24 hr. after spraying suggested that a very high degree of control had been obtained with *p*-nitrophenyl diethyl thionphosphate, rather less with hexaethyl tetraphosphate and an indifferent kill with nicotine. Detailed records of the proportion of aphids killed by the various treatments could not be carried out until 7 days after spraying. Unfortunately, migration of the insects continued during this period, so that the general level of infestation on the control plots (as measured by counts of living aphids per 100 leaves) fell considerably. A detailed statistical analysis of the results was not made as the simple experimental design we were forced to adopt left too few degrees of freedom for error to yield accurate comparisons. It is, however, evident from the data in Table 4 that a high degree of control was obtained with the two organo-phosphorus compounds and a disappointingly low kill with nicotine.

Table 4. *Control of* Brachycaudus helichrysi *with organo-phosphorus compounds*

Treatment	Aphids per 100 leaves	
	Before spraying	After spraying
Hexaethyl tetraphosphate	2132	40
p-Nitrophenyl diethyl thionphosphate	1445	10
Nicotine	1881	250
Unsprayed control	2467	326

Trial 2. *Hop-damson aphid*, Phorodon humuli (*Schr.*)

This trial was carried out on 152 hills of the hop varieties Eastwell Golding and Bramling which, since they did not differ significantly in degree of infestation, may be considered together. Migration of aphids from the wild and cultivated species of

Prunus which constitute the winter hosts was in progress during the latter half of May, and on 8 June 1948, when it was considered that migration was virtually complete, the following four treatments were applied in a randomized block design:

(1) 0·025% hexaethyl tetraphosphate;
(2) 0·025% *p*-nitrophenyl diethyl thionphosphate;
(3) 0·025% nicotine;
(4) unsprayed control.

Population estimates were made immediately before spraying and 48 hr. afterwards and are given in Table 5.

Table 5. *Control of* Phorodon humuli *with organo-phosphorus compounds*

Treatment	Aphids per 100 leaves	
	Before spraying	After spraying
Hexaethyl tetraphosphate	3120	53
p-Nitrophenyl diethyl thionphosphate	2580	5
Nicotine	2630	422
Unsprayed control	2740	2267

Statistical analysis of the complete data showed that the infestation was at first uniformly distributed over the whole trial, but that on the second count the effects of the spray-treatments were highly significant ($P < 0{\cdot}001$). There was not, however, any significant difference between the control obtained with nicotine and that with either of the phosphorus compounds.

Trial 3. *Green apple aphid,* Aphis pomi *Deg.*

The material available consisted of two rows of Malling II apple rootstocks, each 200 yd. long and forming part of a commercially planted stool-bed. The trial was laid out by dividing the material into five blocks each containing four plots, the latter measuring 10 yd. long and two rows wide. The following four treatments were then randomized on this layout:

(1) 0·05% hexaethyl tetraphosphate;
(2) 0·05% *p*-nitrophenyl diethyl thionphosphate;
(3) 0·05% nicotine;
(4) unsprayed control.

Records were made on 7 July 1948 by counting the number of shoots per plot which bore living aphid colonies immediately prior to spraying, and again after periods of 7 and 17 days. Since the rows were open to re-infestation from adjacent stool-beds it was hoped in this way to obtain not only an indication of immediate toxicity of the sprays but also to determine whether any of them possessed sufficient residual toxicity to prevent recolonization. Table 6 gives a summary of the results.

TABLE 6. *Control of* Aphis pomi *Deg. with organo-phosphorus compounds*

Treatment	Colonies per plot		
	Before spraying	7 days after	24 days after
Hexaethyl tetraphosphate	36·0	2·8	20·2
p-Nitrophenyl diethyl thionphosphate	35·8	5·0	14·4
Nicotine	18·4	5·0	17·4
Unsprayed control	25·8	32·8	22·4

Analysis of these data showed that the reduction in colony number after spraying was significant ($0{\cdot}01 < P < 0{\cdot}05$), but again it was impossible to detect a significant difference between the three insecticides. At the end of 24 days, colony numbers were again uniform over the whole trial. It should be noted that the actual reduction in terms of aphids killed was considerably higher than that suggested by the above figures, since the colonies present on sprayed plots after 7 days were all extremely small—being either new colonies formed from immigrants into the experimental area or the remains of what were originally much larger colonies. Similarly, the colonies present on the sprayed plots after 24 days appeared to be appreciably smaller than those on the unsprayed ones.

Taken together, the results of these trials show that the organo-phosphorus compounds are at least as effective in the field control of aphids as the standard nicotine treatment and may even give rather better control. To what extent it is possible to reduce the concentration of organo-phosphorus compound and still maintain adequate control has not been explored in this work, although to judge by some of Schrader's laboratory results, they should prove effective at concentrations considerably lower than those which are possible with nicotine. Although no conclusions may be drawn as to the precise length of time for which *p*-nitrophenyl diethyl thionphosphate confers residual toxicity on a sprayed surface, it is clear from our results with *Aphis pomi* that this must be less than 3 weeks since the plots sprayed with this were, after this lapse of time, infested to about the same extent as those sprayed with the nicotine and hexaethyl tetraphosphate, both of which are known to be fugitive in their action.

The extensive use of potent insecticides like the organo-phosphorus compounds must necessarily be attended by the risk that beneficial insects such as pollinators and predators will be killed equally with the injurious ones. A few observations made in the course of these trials suggest that this danger is likely to be greater with *p*-nitrophenyl diethyl thionphosphate than with hexaethyl tetraphosphate. The former seemed to result in the death of large numbers of coccinellid beetles (*Coccinella septempunctata* L. and *Adalia bipunctata* L.) and of syrphid larvae, both preying on the aphids in trial 1, whereas those sprayed with hexaethyl tetraphosphate still maintained an appreciable predaceous fauna.

We should like to record our appreciation of the assistance given us by Dr R. A. E. Galley of the Agricultural Research Council who supplied us with many of the organo-phosphorus compounds examined. We are also indebted to Mr R. G. Gambrill and Miss V. M. Drew for technical assistance during the course of the work.

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(*Received* 17 *June* 1949)

THE EFFECT OF INSECTICIDES ON RESPIRATION

II. THE EFFECTS OF A NUMBER OF INSECTICIDES ON THE OXYGEN UPTAKE OF ADULT *TRIBOLIUM CASTANEUM* Hbst. AT 25° C.

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(With Plate 8 and 11 Text-figures)

A method is described by which non-volatile contact insecticides may be applied as dusts in a modified Barcroft respirometer with the minimum of disturbance. The technique makes it possible to observe the initial effects of insecticides on insect respiration.

This apparatus has been used to study the effects on the respiration of adult *Tribolium castaneum* Hbst. of a number of insecticides, including so-called inert dusts, and other finely divided solids, some of which are used as diluents.

At 80% R.H. neither talc, carborundum, Almicide (Al_2O_3), stockalite, nor decolorizing charcoal caused any appreciable immediate change in the rate of oxygen uptake of *T. castaneum*, but after 24 hr. the highly toxic substances, Almicide and charcoal, had caused a marked increase. At 20% R.H. talc caused an increase in oxygen uptake only after 48 hr., but Almicide was shown to cause an almost immediate slight increase in respiration and a rapid evolution of water vapour. It is suggested that the increase in respiration may result from an increased metabolism to replace water lost through the cuticle.

3:5-Dinitro-*o*-cresol (D.N.C.), *p*-nitrophenyl diethyl thionphosphate (E 605), γ-isomer of benzene hexachloride (γ-B.H.C.), D.D.T., pyrethrins, toxaphene, chlordane, hexaethyl tetraphosphate all caused an increase in oxygen uptake before killing *T. castaneum* but lauryl thiocyanate did not. Toxaphene and chlordane required a much longer time to cause an increase in respiration than did any of the other substances tested.

D.N.C. in sublethal concentrations caused an increase in the rate of respiration of *T. castaneum*. Concentrations of D.N.C. giving 100% kill caused a rapid increase in oxygen uptake of which the magnitude and duration were independent of concentration.

E 605 caused an increase in respiration after a time delay which was dependent upon the concentration used. The magnitude and duration of the increase in the rate of oxygen uptake were not greatly affected by the concentration of E 605.

The effects of the concentration of γ-B.H.C. and the pyrethrins are complex and require further study. Both substances can affect *T. castaneum* without affecting respiration. Knock-down can occur without affecting the respiration rate.

Introduction

The effects of a number of insecticides on the oxygen uptake of *Oryzaephilus surinamensis* L. have been described by Lord (1949), who used a modified Barcroft respirometer. The experimental technique did not permit the observation of the respiration of the insects until some time after treatment, so that it was possible only

to obtain an estimate of the time required to kill and no measurements of the first changes of oxygen uptake as a result of poisoning were possible. The technique has been modified with the elaboration of an electromagnetic device for the distributing of insecticidal dusts inside the modified Barcroft apparatus without disturbing its thermal equilibrium, and it is now possible to observe the oxygen uptake of insects at all stages after treatment. Using this improved technique a survey of the action of insecticides on insect respiration has been undertaken in order to determine the time required for insecticides to affect respiration and also to determine the correlation, if any, between changes in the rate of oxygen uptake, knock-down and the incidence of death. At the same time the survey has served to demonstrate the kind and intensity of the effects which various insecticides have on *Tribolium castaneum* Hbst., which have been used as the test subjects. The wide scope of the investigation, to date, thus has given a perspective of the type of changes which poisons may cause in the oxygen consumption of insects. As yet it has only been possible to touch on the fringes of the field covering the effects due to the precise method of administration of the poison and also to environment, and therefore it is not possible to discriminate entirely between effects due to the technique employed, and those due to the poison used; this fact has been borne in mind both in the way in which the experiments have been carried out and in attempting to draw conclusions from the work. Nevertheless, a number of very considerable differences in the behaviour of poisons arising out of the concentration of the toxicant have been noted.

Experimental technique

For reasons previously discussed the insecticides have been applied as dusts. The experimental technique used has, however, been developed from that described (Lord, 1949) and differs from it mainly in the use of an electromagnetic device for the application of the insecticide bearing dust to insects inside the modified Barcroft respirometer. Using this apparatus, it is possible to apply non-volatile insecticides as dusts to test insects inside the respirometer without disturbing the thermal equilibrium of the apparatus, and it is therefore possible to make a continuous series of observations which may be commenced before treatment of the insects. Moreover, the insect test subjects are treated rapidly and with the minimum of disturbance. These are both desirable characteristics of the method, since it is important to be able to determine with precision the time at which the poison is applied and to be sure that any changes of respiration of the test subjects are not the result of changes in the environment apart from the application of the toxicant.

The apparatus in its present form does not give a uniform distribution of dust over the whole of the floor of the respirometer vessel. As a result, quantitative application of a dust to test insects is not possible. For the present purposes this limitation has not proved to be a serious drawback, since under the experimental conditions the concentration of toxicant in a dust appears to be far more important than the amount of dust applied (Lord, 1950). Also, if this had not been the case

the experimental results would not have been reproducible, as in fact they proved to be.

Toxicological data have been obtained (Lord, 1950), using dusts made from insecticidal materials tested for their effects on the respiration of *T. castaneum*. These experiments, although not carried out under conditions identical with those obtaining in the respirometers, give an indication of the concentrations at which the insecticides under test become toxic.

Selection of test subjects

As a result of the experimental technique a number of specialized considerations arise in the choice of the test insect. It becomes necessary to choose between the advantages derived from measuring the overall reaction of a relatively large population of small insects and those from a small number of large insects. For the present purposes, it was preferable to obtain the average reaction of a relatively large number of small insects, in order to avoid as far as possible any exceptional reactions which might be peculiar to individuals.

In order to circumvent the difficulty of providing a cage for small insects, which, while confining them, did not at the same time prevent them from being readily dusted, adult beetles of the species *T. castaneum* were used as the test subjects, because they are unable to climb out of clean glass vessels with vertical sides. In addition, *T. castaneum* satisfy the usual conditions required for insects to be useful as test subjects.

Apparatus and methods

Principle of the apparatus. The apparatus is essentially a constant speed motor with the rotor placed inside, and the field coils outside, the respirometer vessel. The rotor incorporates a conical cup which serves to hold the dust, which is thrown out as the rotor turns when the field coils are energized. The dust is then distributed over the whole of the vessel by means of a perforated zinc basket which surrounds the rotor.

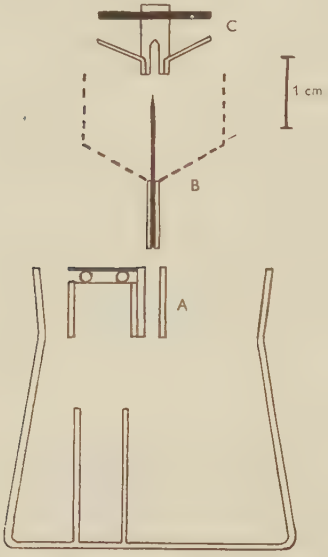
Assembly and use of the apparatus. The functions of the various parts of the apparatus can best be illustrated in conjunction with a description of their assembly. Text-fig. 1 and Pl. 8, fig. 1 show the various parts in the order in which they are fitted together. Pl. 8, fig. 2 shows the various parts assembled, but not in the respirometer vessel.

The alkali is measured on to the filter-paper in the respirometer cup in the usual way and the insects are placed in the apparatus. Then Part A of the apparatus, which serves both as a cover to protect the alkali from the dust and as a support for the rest of the apparatus, is placed in position on the alkali cup. The stem of part B is then fitted into the socket of part A, and part C the rotor, the cone filled with dust, is placed in position on the needle of part B which forms the bearing. The vessel is then fixed on to the manometer, and the apparatus placed in the constant temperature bath and allowed to equilibrate. When it is desired to discharge the dust, the manometer is removed from the constant temperature bath and the electromagnet

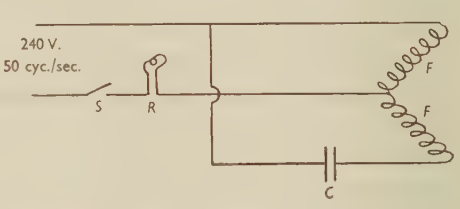
is placed round the vessel and the coils energized; the rotating magnetic field so produced acts on the steel rod in the rotor and spins it. The whole operation takes 15–30 sec. and the respirometer regains thermal equilibrium in a similar time.

Construction of the apparatus. Part A consists of two brass tubes soldered together with their axes parallel; the one forms the socket into which fits the stem of part B, the other fits over the alkali cup of the respirometer vessel.

The end of this latter tube is covered by a metal plate, separated from it by two wires to give an air gap. This prevents dust from falling into the alkali and at the same time does not interfere with its function as a CO_2 absorbent.



Text-fig. 1. Apparatus for distributing dusts inside the Barcroft respirometer.



Text-fig. 2. Wiring diagram of magnets for discharging dusts inside respirometers. *S*, switch; *R*, resistance with indicator lamp; *C*, condenser; *FF*, field coils.

Part B consists of a metal tube which fits into the appropriate tube in part A, and into which is fixed a sewing needle which forms the bearing upon which the rotor (part C) revolves. Part B is completed by a perforated zinc basket, arranged to fit round the rotor. As the dust is flung from the spinning rotor the zinc basket distributes it over the area of the apparatus.

Part C, the rotor, comprises a conical brass cup fitted on to the bearings which allow it to rotate about its axis on the needle of part B. It is rotated by means of a magnetic field acting on the piece of steel wire which is passed through the boss which also forms the bearing.

Rotating magnetic field. The rotating magnetic field was obtained by means of a pair of electromagnets energized from the mains electricity supply (240 V. 50 cyc./sec.). The current through the magnets was set out of phase by inserting a condenser

in series with one of the magnets. A description of the set-up will not be given as the electromagnets were obtained from two old induction motors, all adjustment being made experimentally to obtain the best results from these magnets. The diagram of the circuit is, however, given in Text-fig. 2.

Test subjects. The test subjects used were adult *T. castaneum* Hbst. selected from 3–4-month-old cultures which were reared on whole meal flour at 75° F. and 60% R.H.

Handling and caging of test subjects. The test insects were removed from the culture just before each experiment, using a clean camel-hair paint brush, and were counted into clean uncovered 2 × 1 in. specimen tubes. The insects were then transferred into $\frac{5}{8}$ in. specimen tubes 1 cm. high, which formed the cages in which the insects were confined in the respirometer. Usually twenty-five insects were placed in a tube, and two tubes were placed in each apparatus to give fifty insects per respirometer.

Preparation of dusts. A weighed amount of insecticide was ground by hand in a glass mortar. As the grinding proceeded a weighed amount of the carrier (talc) was added gradually. When the insecticide was a liquid it was weighed into a beaker and absorbed by a small portion of talc; it was then transferred to the mortar for grinding and the rest of the talc was added gradually, each successive portion being used to wash out the beaker.

Dusts containing lower concentrations of the insecticide were then prepared from this dust by diluting it with more talc. Mixing was done in an end-over-end shaker, in order to avoid the progressive reduction of the particle size of solid insecticides which would occur if more drastic methods were used.

Environment of insects under test. All the experiments described here have been carried out at 25° C. In the absence of a rapid evolution or absorption of water vapour the humidity inside the apparatus is controlled by the potassium hydroxide solution used to absorb the carbon dioxide given out by the insects. Unless otherwise stated, a solution of 20 g. of potassium hydroxide in 100 g. of water has been used and under conditions of equilibrium this gives a R.H. of 80% according to Paranjpe (1918–20). At this concentration the resultant humidity only changes slowly with changes in concentration.

The various humidities quoted in the experiments on the action of inert dusts were obtained by using different concentrations of potassium hydroxide solutions: 50 g. of potassium hydroxide in 100 g. of water giving a R.H. of 50% and 100 g. of potassium hydroxide in 100 g. of water giving a R.H. of 20% (Paranjpe, 1918–20).

Selection of carrier. The insecticidal activity of certain so-called inert dusts is well known, and care was therefore taken to select a carrier showing the minimum of toxicity towards the insect species selected for use as test subject.

After a preliminary toxicological trial, summarized in Table 1, the results of which are in line with those described by Alexander, Kitchener & Briscoe (1944), talc was selected as the carrier. However, it has been shown that talc does have a slight action, evident under extreme conditions, and its effect is to increase the rate of

oxygen uptake of *T. castaneum*. In this respect its action appears to resemble that of highly insecticidal 'inert' dusts which also cause an increase in respiration (see Tables 2 and 3).

Presentation of results. The results of the respiration experiments are presented mainly in graphical form, and for clearness, only sufficient experimental points have been plotted to give an accurate representation of the experimental data.

Control experiments were carried out in all cases, but the resultant 'curves' obtained have been omitted from many of the graphs in order to reduce their complexity. This course has been possible as apart from periodic variations about the mean rate of oxygen uptake, the rate of respiration of *T. castaneum* has remained constant throughout the period of the experiments described, and since the respiration of the test subjects prior to treatment has been observed.

Owing to the nature of the experimental results, smooth curves have not been fitted to the data, and the experimental points have been joined by straight lines. In general, this has resulted in fairly smooth curves.

Data to illustrate particular aspects of the action of individual insecticides have been given in tabular form wherever possible, so that information obtained from replicate experiments could readily be compared.

RESULTS

Selection of carrier: the toxicity of 'inert' dusts to Tribolium castaneum. Groups of twenty *T. castaneum* in 2 × 1 in. specimen tubes were placed in glass jars in a constant temperature bath at 25° C. The R.H. in the jars was maintained at approximately 80% by means of a piece of no. 40 Whatman filter-paper soaked in a solution of 20 g. of potassium hydroxide in 100 g. water.

The insects were dusted with portions of the substances under test and were inspected at various intervals, using the controlled temperature warm plate and camel-hair paint brush as described by Tattersfield & Potter (1943). The results are summarized in Table 1.

TABLE 1. *The toxicity of 'inert' dusts to* Tribolium castaneum *at* 25° C. *and* 80% R.H.

Treatment	Percentage mortality														
Talc	0	0	0	0	0	0	5	10	18	33	46	59	69	77	87
Carborundum (600 British Standard grit scale)	0	5	37	46	59	59	66	73	73	83	88	93	95	100	—
Almicide (Al_2O_3)	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Stockalite (china-clay)	0	0	41	51	68	77	90	95	98	100	—	—	—	—	—
Flowers of sulphur	0	0	0	0	0	0	3	10	10	21	28	35	38	50	58
Decolorizing charcoal (B.D.H.)	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Untreated control	0	0	0	0	0	0	0	3	3	5	8	11	24	34	42
Time after treatment in days	1	2	4	5	7	9	11	13	15	18	21	24	26	28	30

The effects of inert dusts on the oxygen uptake of Tribolium castaneum. There appear to be no rapid changes in the rates of oxygen uptake of groups of adult *T. castaneum* on treatment with talc, carborundum, Almicide (a commercial preparation of finely divided Al_2O_3), stockalite (china-clay), flowers of sulphur or decolorizing charcoal at 80% R.H., as is shown in Tables 2 and 3. However, under these conditions of high humidity, the rates of respiration of insects, treated with the highly toxic substances charcoal and Almicide, become much higher than those of the control insects after 24 hr. During this period of time there are no appreciable changes in the rates

TABLE 2. *Effect of inert dusts on the oxygen uptake of* Tribolium castaneum *at* 80% R.H.

(Rates of oxygen uptake, cu.mm./100 insects/min.)

	Time before treatment (min.)		Time after treatment (min.)			After 24 hr. (mean over
Treatment	.90–30	30–0	0–30	30–60	60–90	3 hr.)
	Experiment, 19 February 1948					
None	4·8	4·3	4·7	4·0	4·3	3·8
Talc	4·2	4·0	5·3	5·5	5·5	3·6
Carborundum	3·7	3·2	4·0	5·2	4·3	2·9
Almicide	4·0	4·8	4·1	4·9	4·3	6·2
	Experiment, 5 March 1948					
None	4·0	4·0	2·9	4·3	4·0	2·6
Stockalite	3·6	3·1	4·5	4·3	4·0	3·0
Sulphur	4·0	3·3	3·7	4·1	3·7	2·5
Charcoal	2·6	4·1	3·7	3·3	3·0	4·0

TABLE 3. *Effects of talc and Almicide on the oxygen uptake of* Tribolium castaneum *at various humidities (experiment, 8 March* 1948)

(Rates of oxygen uptake, cu.mm./100 insects/min.)

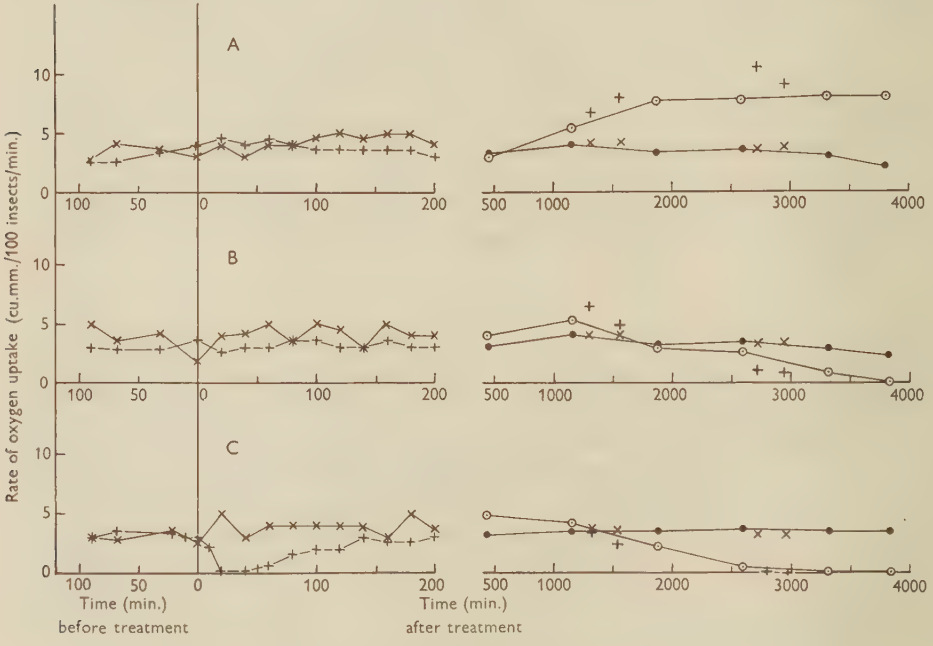
	Time before treatment (min.)		Time after treatment (min.)			After 18 hr. (mean over
Treatment	60–30	30–0	0–30	30–60	70–100	3 hr.)
R.H. 80%:						
None	4·6	5·7	5·0	4·2	5·0	4·2
Talc	4·0	3·1	4·2	4·6	4·6	3·9
Almicide	4·0	3·5	5·4	4·4	4·2	11·2
R.H. 50%:						
None	3·3	5·0	4·0	3·3	3·7	3·7
Talc	4·3	4·0	4·0	4·7	4·3	3·3
Almicide	2·5	2·5	2·3	1·8	—	8·1
R.H. 20%:						
None	3·5	3·2	3·3	3·9	3·9	3·6
Talc	4·2	3·8	3·7	4·2	4·2	3·5
Almicide	3·3	3·8	(1·3)	(0)	(0·5)	1·1

	Time after treatment (hr.)			
	24	42	48	66
R.H. 20%:				
None	3·6	3·3	3·7	3·5
Talc	4·4	7·1	7·5	8·8

of oxygen uptake of untreated insects and those which have been treated with the less toxic substances, talc, stockalite or sulphur.

After prolonged exposure to talc at low humidity the respiration of *T. castaneum* is enhanced (see Table 3). It may be concluded that talc is not without action on *T. castaneum* and that its ultimate action is similar to, though much slower than, the action of Almicide with which it is compared in Table 3.

The action of Almicide at various humidities is shown in more detail in Text-fig. 3, a composite graph derived from two overlapping experiments which have been superimposed.



Text-fig. 3. The effect of Almicide (Al_2O_3) on the oxygen uptake of *T. castaneum* at three humidities.

Experiment, 24 January 1949 (50 adult insects): + Almicide; × untreated control (whole graph).

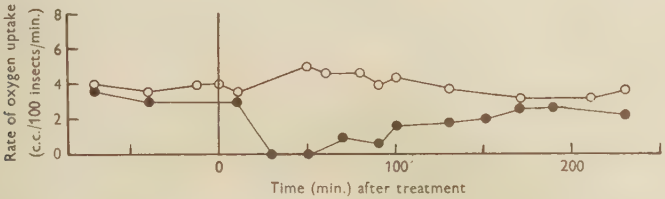
Experiment, 26 January 1949 (10 adult insects): ⊙ Almicide; ● untreated control (later than 500 min. after treatment only).

A, 80 % R.H. B, 50 % R.H. C, 20 % R.H.

If in the first place only the general effects of Almicide on respiration are considered and effects observed in the first 300 min. after the insects have been treated are ignored it can be seen from Text-fig. 3 that the general pattern of action is similar at all humidities. At all these humidities there is a rise in respiration followed by

a fall as the insects die. The increase in the rate of oxygen uptake is slower at higher humidities, but the enhanced rate of respiration is maintained for a longer period at higher humidities where the insects survive for longer than at low humidities.

It has been established by Alexander *et al.* (1944) and Wigglesworth (1944) that inert dusts kill insects by causing a loss of moisture, and hence, since *T. castaneum* feeds on flour, of which the moisture content is well below 20%, and other cereal products, and seems capable in its natural environment of living normally under conditions of low humidity, it is likely that its water content is maintained, at least in part, by the oxidation of its food. It is possible that the metabolism of insects treated with an inert dust increases in order to replace water lost through the cuticle by oxidation of metabolites to give water and other products such as CO_2. On this basis it is also possible to account for the prolonged period of enhanced oxygen uptake of *T. castaneum* treated with Almicide and kept at 80% R.H., since under these conditions the rate of water loss is much smaller than at low humidities and therefore the moisture produced by increased metabolism would be more effective in delaying the drying up of the insects.



Text-fig. 4. The effect of Almicide on the oxygen uptake of *T. castaneum* at low humidity.

●—● KOH solution only; ○—○ KOH solution plus solid KOH.

As shown in Table 3 and Text-fig. 3 C at 20% R.H. the rate of oxygen uptake of *T. castaneum* apparently rapidly falls almost to zero when the insects are treated with Almicide. However, on the inclusion of an additional drying agent such as solid potassium hydroxide inside the respirometer (see Text-fig. 4) no such fall in the apparent oxygen uptake occurs; it may therefore be concluded that the apparent cessation of oxygen uptake is due to a sudden and rapid evolution of water vapour, which is not absorbed by the potassium hydroxide solution sufficiently rapidly to give a true value of the oxygen uptake.

At the low humidities maintained by the use of additional drying agents, groups of insects show slightly increased rates of oxygen uptake after treatment with Almicide (Table 4). In contrast to this the rate of respiration of untreated control insects (maintained at 20% R.H.) shows a tendency to fall (Table 4). It may therefore be concluded that Almicide causes a definite, although slight, increase in oxygen uptake a short time after it is applied.

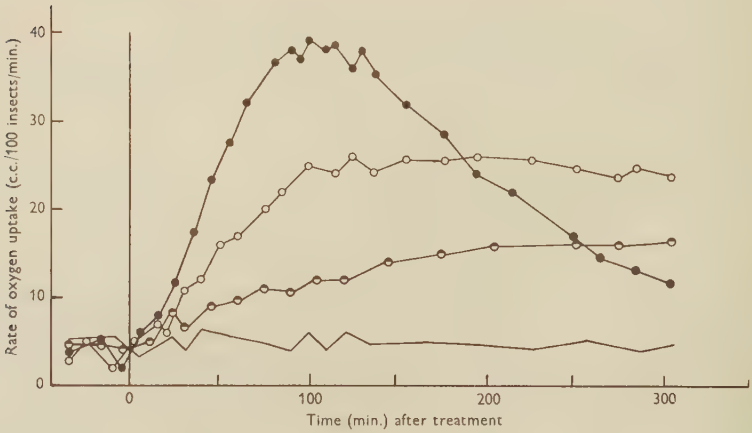
TABLE 4. *The effect of Almicide on the oxygen uptake of* Tribolium castaneum *at low humidity*

(Mean rates of oxygen uptake, cu.mm./100 insects/min. Experiment, 8 February 1949. Insects treated with Almicide. CO_2 absorbent, 0·4 ml. of 100 g. KOH in 100 g. water.)

	Time before treatment (min.)		Time after treatment (min.)	
Extra drying agent	130–70	70–10	10–70	70–130
None	5·7	4·8	1·8	3·0
Potassium hydroxide (solid)	3·8	3·7	5·0	5·3
Calcium chloride (fused)	4·7	4·3	4·7	5·7
Phosphorus pentoxide	4·5	3·8	4·7	5·6
Zinc chloride (fused)	4·7	2·8	4·5	5·5

Untreated control insects

	Approximate time from beginning of experiment (min.)			
Date of experiment	0–60	60–120	140–200	200–260
8 February 1949	4·8	4·3	3·7	3·3
8 March 1948	3·3	3·1	2·9	2·5
24 January 1949	3·3	3·2	4·2	3·7
3 February 1949	3·8	3·8	3·5	2·8



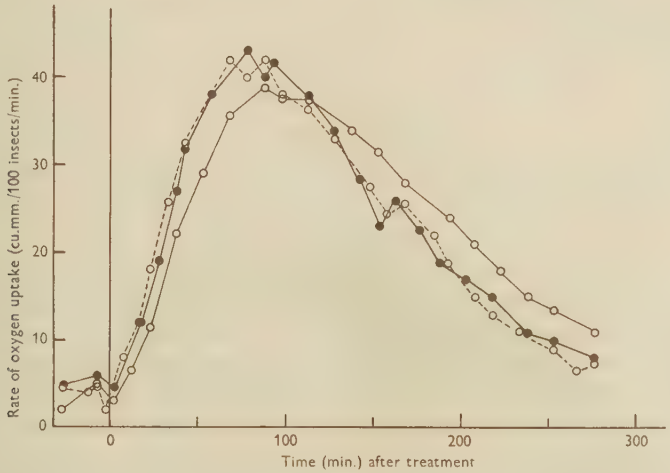
Text-fig. 5. The effects of D.N.C. on the oxygen uptake of *T. castaneum*.

Mortality after 24 hr.:
— Control, 0 %; ● 0·25 % D.N.C., 94 %;
○ 0·175 % D.N.C., 32 %; ◓ 0·125 % D.N.C., 2 %.

The effect of 3 : 5-*dinitro*-o-*cresol.* The general effect of D.N.C. is to cause a rapid initial increase in the rate of oxygen uptake of adult *T. castaneum*, which is followed by a fall in respiration as the insects die. The magnitude of the effect is dependent upon the concentration of D.N.C. in the dusts applied, the increase in oxygen

uptake being correlated with the mortality observed. Sublethal concentrations also cause enhanced oxygen uptake; this is illustrated in Text-fig. 5 by the effect of the 0·125% dust which caused a considerable increase in the rate of oxygen uptake, but which nevertheless caused a 2% kill after 24 hr., when the rate of oxygen uptake of the insects was still between two and three times that of normal insects. Similarly, the 0·175% dust causing a 32% mortality increased respiration beyond that to be expected, if the respiration of only those insects killed by the dust was affected.

Increasing the concentration of D.N.C. in dusts beyond the minimum concentration giving a 100% kill does not appear to change the effects on respiration (see Text-fig. 6), so that a higher concentration neither has a more rapid nor intense effect.



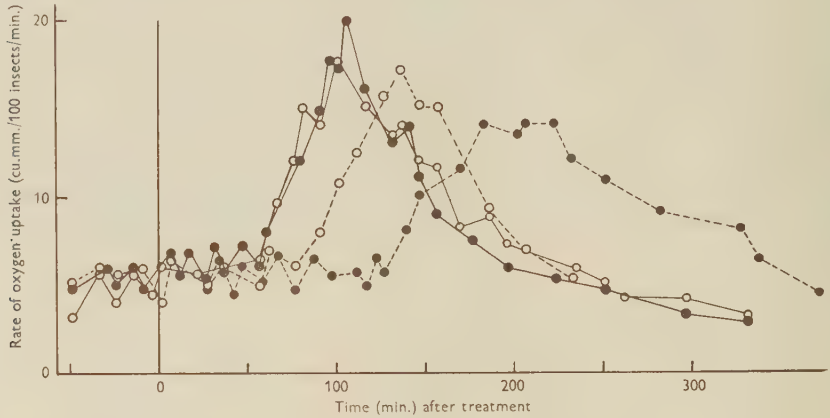
Text-fig. 6. The effect of lethal concentrations of D.N.C. on the oxygen uptake of *T. castaneum*.

O- - - -O 5 % D.N.C.; ●——● 1 % D.N.C.; O——O 0·25 % D.N.C.

The effect of p-*nitrophenyl diethyl thionphosphate.* As can be seen from Text-fig. 7, the general effect of E 605 is to cause an increase of oxygen uptake of *T. castaneum* some time after application. This increase of oxygen uptake is associated, as shown by visual inspection, with the cessation of movement of the insects and is followed by a slow decline in the rate of oxygen uptake.

In addition, Text-fig. 7 shows that the major effect of increasing the concentration of E 605 in a dust is to increase the speed at which the poison acts, rather than to affect the intensity of its action. A second effect, also noticeable in Text-fig. 7, is that as the time for the insects to be affected increases with the dilution of the E 605, so the maximum value of rate of oxygen uptake appears to decrease, and at the same time changes in the rate of respiration appear to occur more slowly. This may

be due to individuals succumbing over a longer period of time at the lower concentrations of E 605, so that the net effect is to give a smaller increase maintained for a longer time.



Text-fig. 7. The effect of E 605 on the oxygen uptake of *T. castaneum*.

○——○ 2·5 % E 605; ●——● 0·5 % E 605;
○- - - -○ 0·1 % E 605; ●- - - -● 0·02 % E 605.

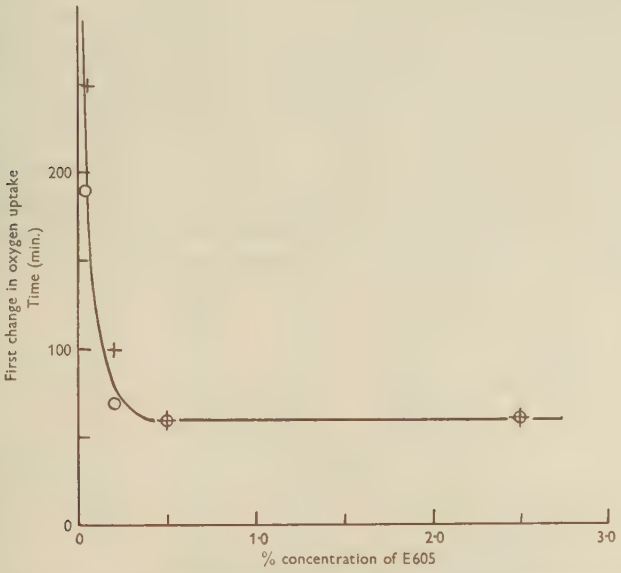
The time required for the rate of respiration to begin to increase (lag period) may be considered as a measure of the rate of action of the poison, and if this is plotted against time (Text-fig. 8) it can be seen that increasing the concentration of poison beyond 0·5% causes little increase in the speed of action, but the time taken for any toxic manifestation to occur increases rapidly when the concentration is reduced below this level.

The effect of γ-isomer of benzene hexachloride. The effects of γ-B.H.C. on the oxygen uptake of *T. castaneum* can be seen from Text-fig. 9 to be complex, and highly dependent upon concentration.

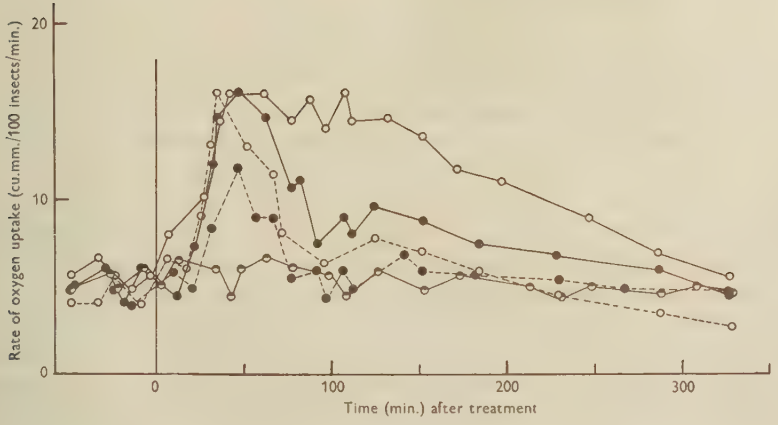
The lowest concentration 0·002% which gave a 64% kill after 24 hr. appeared to have no effect on the respiration of the *T. castaneum*. The other more concentrated dusts used, which all gave a complete kill within 24 hr., all gave initial increases in oxygen uptake. Although the three most concentrated dusts gave approximately equal increases in the rate of respiration, the period of time for which the enhanced respiration continued appeared to be greater the higher the concentration of γ-B.H.C. This phenomenon may arise from the fumigant properties of γ-B.H.C. or may be due to γ-B.H.C. having a secondary action at high concentrations, but further work is required to elucidate this.

When an increase in oxygen uptake occurred, it appeared to be associated with the first visible symptoms of poisoning.

The effect of pyrethrins. The pyrethrins were applied in the form of a dust, pre-



Text-fig. 8. The effect of concentration of E 605 on the time required for the oxygen uptake of *T. castaneum* to be affected.

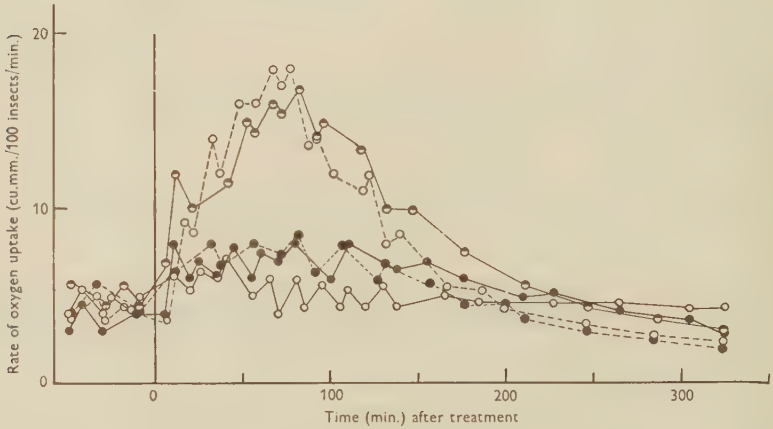


Text-fig. 9. The effect of γ-B.H.C. on the oxygen uptake of *T. castaneum*.

○——○ 5·0 % γ-B.H.C.; ●——● 0·5 % γ-B.H.C.; ○- - -○ 0·25 % γ-B.H.C.
●- - -● 0·01 % γ-B.H.C.; ◓——◓ 0·002 % γ-B.H.C.

pared by absorbing a solution of pyrethrins in petroleum ether (b.p. 40–60° C.) into talc. The solution contained 1·86% pyrethrin I, 1·15% pyrethrin II, as estimated by the mercury-reduction method. The total solids content of the solution was 3·3%. The pyrethrins were prepared from a commercial concentrate by means of the nitromethane-extraction technique described by Barthel, Haller & LaForge (1944).

As in the case of all the insecticides described above, the pyrethrins caused an increase in the oxygen uptake of *T. castaneum* when applied in suitable amounts (see Text-fig. 10). Any increase in oxygen uptake occurred very quickly after treatment and at about the same time as the insects were knocked down. However,



Text-fig. 10. The effect of pyrethrins on the oxygen uptake of *T. castaneum*.

○——○ 3·0 % pyrethrins; ◓——◓ 1·0 % pyrethrins;
●- - - -● 0·3 % pyrethrins; ●——● 0·1 % pyrethrins;
○— —○ 0·03 % pyrethrins.

knock-down also occurred at an early stage in the cases where no increased oxygen uptake occurred, so that there appears to be no correlation, in this case, between the first visible signs of action and the effects on the oxygen uptake.

It is interesting to note the similarity between the oxygen uptake of insects poisoned with different concentrations of pyrethrins once the initial increased respiration has fallen off. This takes place approximately 150 min. after treatment (see Table 5). At this stage the oxygen uptake of the poisoned insects is very similar, although previously the rates of respiration have been widely different, depending on the concentration at which the pyrethrins were applied. Since the insecticide known as 'the pyrethrins' is a complex mixture of active principles which may have different biological activities it is tempting to suggest that the various effects of the 'pyrethrins' on the respiration of *T. castaneum* are due to the separate constituents,

a point which can be settled by work using the pure components of the mixture constituting the natural pyrethrins.

TABLE 5. *Oxygen uptake of* Tribolium castaneum *treated with pyrethrin dust*

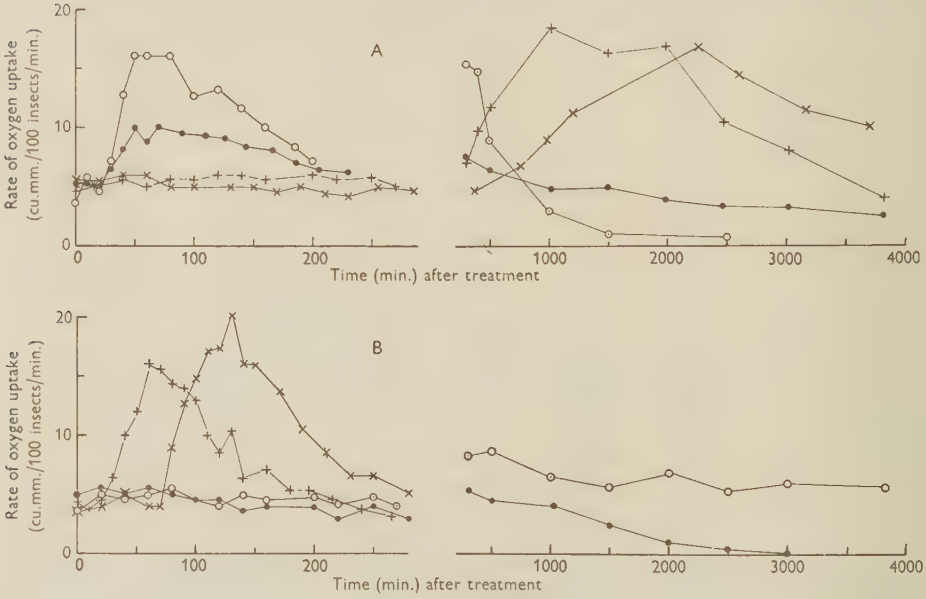
(Rate of oxygen uptake, cu.mm./100 insects/min.)

Percentage concentration of pyrethrin dusts	Time after treatment (min.)					
	60	120	150	180	210	240
	Experiment, 28 September 1949					
3·0	17·5	13·0	8·7	6·2	4·5	4·4
0·15	6·5	8·2	7·2	6·2	5·0	4·0
0·10	6·2	7·8	8·5	7·2	5·5	4·4
0·075	7·2	7·0	6·5	5·5	5·2	4·8
0·05	5·8	6·3	6·3	5·2	6·0	5·4
Control	4·8	5·0	4·7	4·2	4·5	4·2
	Experiment, 6 October 1948					
3·0	16·2	9·9	7·0	5·5	4·4	3·6
1·0	11·0	8·2	5·5	4·0	4·0	3·0
0·3	9·5	8·0	6·2	4·6	4·0	3·2
0·1	7·0	8·0	6·0	5·0	4·6	4·0
0·03	4·5	3·4	4·0	3·8	3·4	3·6
Control	4·5	4·0	4·0	4·4	3·4	4·0
	Experiment, 8 October 1948					
3·0	17·0	10·0	7·0	5·3	4·2	3·6
1·0	15·2	12·5	9·0	7·0	5·8	4·6
0·3	8·0	6·6	5·8	4·8	3·6	3·2
0·1	6·8	7·2	7·0	6·0	5·0	4·6
0·03	4·5	4·4	5·0	4·6	4·6	4·6
Control	4·7	4·4	5·0	4·0	5·4	4·0

The effects of a number of insecticides on the oxygen uptake of Tribolium castaneum *at concentrations giving* 100% *kill.* The oxygen uptake of *T. castaneum* treated with D.D.T., γ-B.H.C., toxaphene, lauryl thiocyanate, H.E.T.P. (hexaethyl tetraphosphate) and E 605 at single concentrations in talc which give 100% mortality are shown in Text-fig. 11. The insect respiration curves, 300 or more minutes after treatment, are all derived from a single experiment (8 February 1949). The ten insects used in each respirometer were dusted by hand as each apparatus was set up.

The curves showing the earlier stages of poisoning (0–300 min.) were obtained in a number of other experiments (D.D.T., 23 February 1948; chlordane, lauryl thiocyanate and control, 26 August 1948; toxaphene, 27 August 1948; γ-B.H.C., 4 October 1948; H.E.T.P. and E 605, 7 May 1948). With the exception of the experiment on D.D.T., when 100 insects were used, the observations were made on groups of fifty *T. castaneum.* The poisons were applied by means of the electromagnetic device previously described. Only qualitative comparisons are possible, since to date, insufficient information has been accumulated to make quantitative comparisons justifiable.

The increase in the rate of respiration caused by a 25% D.D.T. dust is less than that caused by a 5% dust of γ-B.H.C. (see Text-fig. 11A), but the rise in oxygen uptake occurs after about the same time for both insecticides. However, insects treated with D.D.T. respire for a longer period than those treated with γ-B.H.C. In contrast with these poisons which affect the insects shortly after treatment, chlordane and toxaphene cause an increase in the respiration of the insects a considerable time after application. The time-lag for the insects to be affected in the case of chlordane and toxaphene may be associated, as in the case of E 605, with



Text-fig. 11. The effects of a number of insecticides on the oxygen uptake of *T. castaneum*.

A. ●——● 25 % D.D.T.; ⊙——⊙ 5 % γ-B.H.C;
+——+ 2·5 % chlordane; ×——× 2·5 % toxaphene.
B. +——+ 2·5 % H.E.T.P.; ×——× 2·5 % E 605;
●——● 2·5 % lauryl thiocyanate; ⊙——⊙ control, talc.

concentration, but as the concentration at which each of these insecticides has been applied is 2·5%, considerable increases are not possible and it would therefore appear that these poisons are very slow in their action on *T. castaneum*.

The action of H.E.T.P. on the oxygen uptake of *T. castaneum* at the same concentration (2·5%) as E 605 is similar (Text-fig. 11B), although the increase in respiration occurs more quickly after treatment. This is in line with the assumption of similar modes of action for the two poisons. In contrast with all of the other

substances tested lauryl thiocyanate does not give any increase in oxygen uptake before the death of the insects.

Discussion

The work described here is intended as a survey of the types of action which insecticides may have on the oxygen uptake of insects. Neither the list of substances used nor the experiments carried out on any particular insecticide are exhaustive, but a number of phenomena enumerated below have been observed, which should provide a useful background against which more detailed investigations of the action or specific aspects of the action of any particular insecticide may be carried out.

(*a*) *So-called 'inert dusts'*. The action of a number of 'inert dusts' was investigated to facilitate the choice of a carrier with minimum of action on the test insects. Talc was eventually selected as carrier.

The action of all the 'inert dusts' tested was to cause an increase of oxygen uptake which was associated with the toxic action of the dust. In the case of Almicide (a proprietary preparation of Al_2O_3) which was investigated more thoroughly, it was shown that the insects were killed more rapidly the lower the humidity. It was also demonstrated that at low humidities water vapour was rapidly evolved on treatment with Almicide, and that this was associated with an increase in respiration. A possible explanation of the increased oxygen uptake of affected insects is that the animals attempt to replace water losses through the cuticle by water, derived from metabolic sources.

(*b*) *The effect of insecticides on the oxygen uptake of* Tribolium castaneum. Most of the insecticides tested caused an increase in oxygen uptake at some stage before death. Lauryl thiocyanate was the only exception, although it should be noted that rotenone and Lethane B 71, which are not toxic to *T. castaneum* under the experimental conditions, do not cause an increase in respiration before killing *O. surinamensis* (Lord, 1949).

(*c*) *Time-lag of effects*. Of the substances tested at a concentration of 2·5% (and giving 100% mortality), chlordane and toxaphene are outstanding for the long time elapsing before any increase in oxygen uptake occurs. The only other appreciable time-lag occurred in the case of E 605.

(*d*) *The effects of concentration on the action of E* 605, *D.N.C., γ-B.H.C. and pyrethrins*. The time at which the rise in oxygen consumption of *T. castaneum* treated with E 605 occurs is dependent upon the concentration of E 605. The higher the concentration the sooner the increase until a certain concentration is attained when further increases do not reduce the time-lag after treatment. This does not appear to hold for D.N.C., γ-B.H.C. or the pyrethrins, although changes in the concentration of these poisons are not without other effects. γ-B.H.C. and the pyrethrins at their lowest toxic concentrations apparently kill without causing any very considerable changes in oxygen uptake, whilst higher concentrations of these poisons cause increases in oxygen uptake of *T. castaneum* within certain limits. The effect of the pyrethrins, however, differs from that of γ-B.H.C. at higher concen-

trations, in that the higher the concentration of γ-B.H.C. the more prolonged the period of enhanced oxygen uptake, whilst the period of enhanced oxygen uptake caused by the pyrethrins does not appear to be dependent upon the concentration of the pyrethrins. The effect of D.N.C. is again different, since this substance can increase the rate of respiration of *T. castaneum* without killing. Higher concentrations of D.N.C. result in higher rates of oxygen uptake associated with some kill. The rate of respiration increases with concentration until a complete kill is obtained. Further increases in the concentration of D.N.C. appear to have little additional effect on the rate of oxygen uptake or the rate at which it changes.

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EXPLANATION OF PLATE 8

Fig. 1. The dusting apparatus for use inside a modified Barcroft manometer. The parts are in order of assembly.

Fig. 2. The dusting apparatus for use inside a modified Barcroft manometer. Assembled as for use inside the manometer, with a respirometer vessel for comparison.

(*Received* 28 *July* 1949)

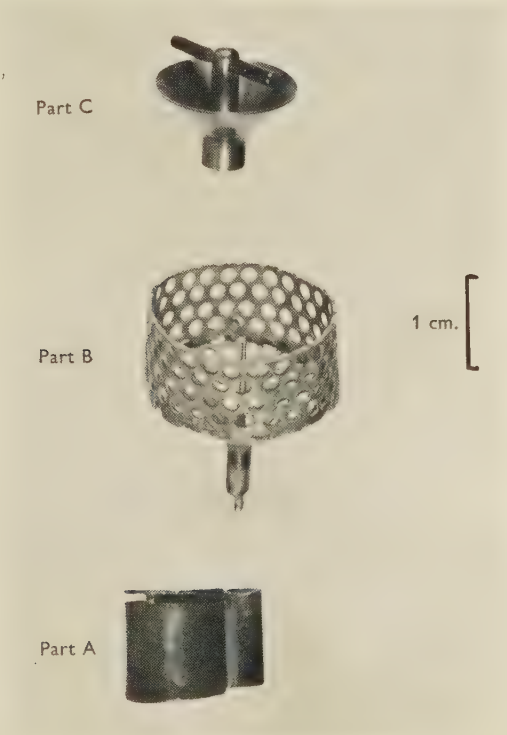

Fig. 1

Fig. 2

LORD—*Effect of insecticides on the respiration of* Tribolium castaneum

THE EFFECTS OF CONCENTRATION OF POISON AND THE AMOUNT APPLIED ON THE TOXICITY OF INSECTICIDAL DUSTS TO ADULT *TRIBOLIUM CASTANEUM* Hbst.

By K. A. LORD

Rothamsted Experimental Station, Harpenden, Herts

Using insecticidal dusts of D.D.T., γ-benzene hexachloride and D.N.C. under the experimental conditions, comparatively large changes in the amount of material of a given concentration are necessary in order to give appreciable differences in mortality, but small changes in the concentration of the poison will produce large differences in toxicity with a given amount applied. This relationship would appear to hold with a number of insecticides.

Under the experimental conditions described in the paper on the effect of insecticides on respiration by Lord (1950) the concentration of poison in a dust could be accurately controlled, but the amount of material applied to the insects could not, therefore a short series of experiments to discover the relative importance of these two factors influencing toxicity was carried out, under similar conditions. Talc was used as a carrier because it had been found to have very little effect on the test subjects used.

The experiments have been divided into two groups: in the first the influence on toxicity of the amount of an insecticidal dust has been compared with that of the concentration of the poison; in the second the effect of changes in concentration on the toxicity of an insecticide when approximately equal amounts of dust have been applied was examined, using a number of different poisons.

Test subjects

The insects used were adult *Tribolium castaneum* Hbst. reared on wholemeal flour in a constant temperature room maintained at 75° F. and 60% R.H. Just before treatment the insects were removed from culture and counted into 2 × 1 in. specimen tubes.

The insects were inspected using a controlled temperature warm plate (27–40° C.) (Tattersfield & Potter, 1943) and a camel-hair paint brush. They were separated into the categories: N, normal; S, slightly; B, badly affected; M, moribund; D, dead. The mortality was then assessed as the fraction of the total number of insects which were B, M and D.

Comparison of the effect on mortality of concentration of insecticide and the amount of dust applied

Experimental method. Portions of the appropriate insecticide containing dusts were carefully weighed out into 2 × 1 in. specimen tubes. Groups of twenty adult

T. castaneum were placed in the tubes which were then gently shaken in order to distribute the dusts over the insects. The insects were then kept in contact with the dusts in the specimen tubes under controlled conditions of temperature and humidity until they were inspected. Three replicates were used.

TABLE 1. *Effect of amount of dust applied and concentration of 3 : 5-dinitro-o-cresol on toxicity*

(Experiment, 7 March 1949 (mortality after 24 hr.). Test subject, *Tribolium castaneum*. Temperature, 58° F. R.H. 55 %.)

Weight of dust applied (mg.)	% kill	% kill	% kill	% kill	% kill
20	—	10	38	80	93
40	3	26	57	93	97
80	2	29	80	98	—
160	0	37	95	90	—
320	3	—	—	—	—
Percentage concentration of D.N.C.	0·175	0·25	0·35	0·5	0·7

Mortality of insects treated with talc was zero at all levels of application.

TABLE 2. *Effect of amount of dust applied and concentration of γ-isomer of benzene hexachloride on toxicity*

(Experiment, 12 March 1949 (mortality after 48 hr.). Test subject, *Tribolium castaneum*. Temperature, 58° F. R.H. 58 %.)

Weight of dust applied (mg.)	% kill	% kill	% kill	% kill	% kill
20	5	37	80	93	100
40	27	42	83	98	100
80	35	68	92	100	100
160	41	67	95	100	100
Percentage concentration of γ-B.H.C.	0·0025	0·005	0·01	0·02	0·05

Mortality of insects treated with talc was zero at all levels of application.

TABLE 3. *Effect of amount of dust applied and concentration of D.D.T. on toxicity*

(Experiment, 28 April 1949 (mortality after 24 hr.). Test subject, *Tribolium castaneum*. Temperature, 70° F. R.H. 60 %.)

Weight of dust applied (mg.)	% kill	% kill	% kill
20	2	63	100
60	2	75	100
180	7	73	97
Percentage concentration of D.D.T.	0·01	0·04	0·2

Mortality of insects treated with talc was zero at all levels of application.

TABLE 4. *The toxicity of a number of insecticides as dusts to* Tribolium castaneum

Substance	Percentage concentration	Percentage mortality (24 hr.)	Percentage mortality (48 hr.)
(Experiment, 26 July 1948. Temperature, 75° F. R.H. 70%.)			
D.N.C.	1·0	98	98
	0·5	67	67
	0·25	24	24
	0·125	0	0
E 605	0·025	100	100
	0·0125	100	100
	0·00625	7	92
	0·003125	0	1
Lauryl thiocyanate	1·25	100	100
	0·625	97	100
	0·3125	22	13
	0·25	4	2
Chlordan	0·25	100	100
	0·10	99	100
	0·05	93	99
	0·025	51	79
	0·0125	9	32
	0·00625	1	5
Toxaphene	2·5	100	100
	1·0	100	100
	0·5	87	100
	0·25	74	99
	0·125	51	82
	0·0625	1	20
D.D.T.	25·0	100	100
	5·0	93	100
	1·0	90	96
	0·2	86	86
	0·04	6	5
γ-B.H.C.	0·05	99	100
	0·02	90	90
	0·01	78	64
	0·005	36	11
	0·0025	12	0
	0·00125	0	0
(Experiment, 14 September 1948. Temperature, 75° F. R.H. 70%.)			
Pyrethrins	0·3	100	100
	0·15	100	100
	0·10	92	88
	0·075	88	78
	0·05	0	8
	0·03	0	0
Controls talc (both dates)	—	0	0

Results. The results have not been subjected to statistical treatment, since the effects of changes of concentration of D.N.C., γ-B.H.C. and D.D.T. can be

readily seen by inspection of Tables 1–3 to be much greater than those due to corresponding changes in the amount of dust applied. Examination of the results by the method of probit planes (Finney, 1947) would not assist our present purposes.

It should be noted, however, that in the cases of D.N.C. and γ-B.H.C., where sufficient data are available, that provisional probit lines can be drawn which show no considerable deviations from linearity.

Since the effect of concentration is so much greater than that of the amount of dust, the quantity of dust applied to the test subjects need not be regulated with great precision, when the critical concentration of insecticide in a dust is being assessed under the experimental conditions described here.

Comparison of a number of insecticides as dusts

Experimental method. Portions of insecticidal dusts, judged to be between 50 and 100 mg., were applied to counted groups of *T. castaneum* in 2 × 1 in. specimen tubes. The tubes were gently shaken to distribute the dust and were then stored under controlled conditions of temperature and humidity until the insects were inspected. Five replicates were used.

Results. The results set out in Table 4, show, in general, that a relatively small increase in concentration of poison in a dust results in a large increase in mortality. Frequently a fourfold increase in concentration has resulted in a change from a very low to very high mortality. As a result it has not been considered worth while to carry out a full statistical examination of the results, although in most cases only small deviations can be observed when provisional probit lines are plotted.

Duplicate sets of experiments have been made but have not been reported, since they are in agreement with the experimental results given, and provide no additional information.

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(*Received* 28 *July* 1949)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Thursday, 6 October 1949, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

The following papers were read and discussed:

1. Virus diseases of stone-fruit trees. By Dr R. S. Willison.
2. Virus diseases of cacao. By Mr A. F. Posnette.

Short notes were read by Mr S. H. Crowdy, Dr L. C. Luckwill and Dr I. W. Prentice.

VIRUS DISEASES OF STONE-FRUIT TREES

By R. S. Willison

I would like, first of all, to express my appreciation of the honour of being asked to take part in to-day's discussion. I really am grateful to the association and its council for the opportunity of presenting some account of our observations on the viruses of stone-fruits in Ontario, and a few remarks on the general picture that is gradually being pieced together from the jig-saw puzzle pieces scattered over various parts of the North American Continent.

To carry the metaphor a little further, one might add that we are still in those early stages of the puzzle, when a few pieces have been fitted together here and there, and a good many more pieces are face up but do not seem to belong anywhere. It is also extremely probable that there are still plenty of pieces waiting to be turned up. For a further complication, this puzzle is neither a one-man nor a one-team game and the participants have naturally been most intent on their own little sections, though frequently casting a roving and speculative eye over other parts of the board.

Progress is necessarily slow, partly because different matching pieces in the puzzle may turn up in Washington, British Columbia, Wisconsin or Ontario, and even more because it takes so long to find out if the piece even belongs in the picture and then to see where it might fit.

As you probably know, there have been several occasions when American and Canadian workers have got together to take stock of the general situation, to make some suggestions for integration of methods of attack and to try to establish fairly close, even if unofficial, liaison between the various working parties. In some respects, these attempts have met with considerable success, though in others progress has been much slower.

It should be borne in mind that, for obvious geographical and climatic reasons, natural communities, in North America as elsewhere, ignore political considerations and the international boundary, so that affinities between plant diseases, among other things, tend to be stronger across than along the border. Consequently there are, or have been, more interests in common between the plant pathologists of British Columbia and the Pacific States on the one hand and between those in Eastern Canada and the Great Lakes States on the other, than there is, say, between British Columbia and Ontario, or between Michigan and Oregon. Recent developments give some indications that the virus diseases of East and West may not be quite so different as was at first supposed. On the Canadian side of the border, at least,

a standing committee at research level was formed last year to facilitate more rapid exchange of information and ideas between East and West and to promote ways and means of determining and surveying common ground.

Back in 1941, one of the first moves towards concerted action was made when a conference of pathologists from the Great Lakes States and Eastern Canada met in Michigan, under the leadership of Prof. V. R. Gardner, Director of the Michigan Agricultural Experiment Station. One important practical outcome of that conference was the setting up of a committee charged with compiling the information then available in what is now known as the *Handbook of Virus Diseases of Stone-Fruits in North America*, published in 1942. This same conference also set in motion plans for co-ordinating and integrating future research as far as it could feasibly be done. To this end, they made several suggestions, such as:

(i) The adaption of a *standard* minimum range of differential hosts, which would be of great value in identifying the disease occurring in different districts.
(ii) Uniformity in ways and means of indexing stock and scion sources to determine their virus status.
(iii) The establishment of certification schemes for nursery stock and so on.

A similar conference, held in the Pacific North-west in 1945, followed suit by recommending:

(i) The use of a standard differential host range.
(ii) The expediting of vector research.
(iii) A uniform plan for nursery certification.
(iv) State conducted control and eradication measures backed by extension (or educational) programmes to develop grower co-operation.

Since then, contacts between East and West have become a little closer, with the formation of a committee of workers drawn from both areas and from the Federal departments of Agriculture of both the United States and Canada. This committee has just finished preparing the *Handbook* for republication in revised and illustrated form. The revised *Handbook* is now in the press and should be ready for distribution within the next few months. In this revision, special efforts were made to have the new manual as authoritative as it could be. Thus the report on each disease was prepared, reviewed and approved by a subcommittee composed of every individual known to be working with that particular disease. Since much of the information has not been published elsewhere and has been freely contributed by the various investigators concerned, the completion of the *Handbook* revision is a tribute to the generous co-operation of individual pathologists, as well as to the zeal, patience and generalship of the editorial committee, both individually and collectively. Unfortunately, such a publication, in the very nature of things, cannot profess to be the final or even the latest word on the subject. But it should be a very useful mine of information for all those interested in the stone-fruits and their virus diseases. A supplementary section deals with troubles which are due to other causes, and whose symptoms might be confused with or superimposed on those of the virus diseases.

Up to the present, work on the stone-fruit viruses has been largely exploratory and attention in both East and West has been devoted principally to what may be termed the more practical aspects of the problem. First, of course, has been the demonstration of transmissibility or the determination of the virus origin of the various disorders not definitely assignable to other causes; secondly, the study of symptom expression in the host plants affected; thirdly, the development of indexing procedure and the search for virus-free sources of different varieties from which to build up foundation stock; and fourthly, the establishment of state and provincial certification of nursery stock for freedom from at least the more commonly occurring and destructive viruses.

A good deal of interspecific and inter-varietal cross-inoculation is being carried on at many of the stations, and this work is gradually unravelling some of the tangled skein of identity of the causal viruses as well as adding to our knowledge of symptomatology. But the use of a standard minimum range of differential hosts, as recommended by both the Eastern and the Western Conferences, has not yet been generally adopted. For that matter, there is not

complete unanimity as to what varieties should constitute such a standard range, though the ones used in several places in the East, namely peach, the sour cherry variety Montmorency, and the sweet cherry varieties, Black Tartarian and/or Napoleon, and Italian prune, are in the main accepted as a starting-point.

Before going on to discuss individual diseases, a few general remarks about some of the peculiarities of the symptom expression of stone-fruit viruses might be in order.

Many of them pass through two distinct phases, characterized by what have been termed 'shock' and 'chronic' symptoms (which are not quite the same as the 'primary' and 'systemic' symptoms of the tobacco and cucumber diseases).

Shock symptoms are usually acute, and are thought to be the result of the reaction of *active healthy* meristematic tissue to virus invasion, i.e. they appear on developing organs which have become infected *after* they have started to grow. Consequently shock symptoms almost invariably appear only in the beginning of the first growing season after infection. (Large trees are exceptions because it may take 2–3 years for disease to become systemic in them.) Shock symptoms are non-recurrent, though there are some intermediate types which are severe during the shock phase, but may recur spasmodically in milder form during the chronic phase (for example the 'etching' of necrotic ring spot).

Chronic symptoms are, on the other hand, recurrent, appearing in organs which are already infected before starting to grow. Chronic symptoms may be consistently severe, or mild, or vary in severity from season to season. Some may be present only on leaves, developing during the early part of the season or others may not appear until late in the growing season. Some are persistent, some evanescent, and so on.

Some diseases, for example necrotic ring spot of sour cherry, may have shock symptoms but no definite chronic ones, i.e. the disease tends to become masked.

Some, for example *X*-disease of peach, peach yellows, line pattern, have chronic symptoms, but no distinctive shock phase.

Some, for example sour cherry yellows, and tatter leaf of sweet cherry, have both types of symptoms.

Some diseases have one of these combinations on one host and a different combination on another host.

Finally, there are some hosts which approach the true carrier type, being capable of becoming infected with disease, but showing no recognizable symptoms of either type, for example some plums with prune dwarf or necrotic ring spot.

It is also just as well to bear in mind that under certain conditions shock symptoms can be suppressed, for example:

(i) If inoculation is made by the usual nursery procedure of budding in late summer, and cutting back to the bud in the spring.

(ii) If certain varieties, for example Montmorency (sour cherry), are inoculated late enough in the growing season for growth to have stopped but early enough to allow the virus to become systemic before leaf fall.

(iii) Shock symptoms may be suppressed or delayed in peach by inoculating in the spring after growth has started.

Young Montmorency trees respond with the most striking shock symptoms when they are inoculated just before bud-break (Jones's budding). On the other hand, peach seedlings grown outdoors and inoculated at the normal budding time (late August or early September) go into shock the following spring. In both instances the tops should be left on.

Chronic symptoms, on the other hand, usually appear sooner if the inoculated plant is topped in the usual way. They may frequently be induced a few weeks after inoculation, early in the growing season, if the tops are cut back and the plants are kept under conditions which force rapid growth.

Our usual practice is outdoor inoculation in late summer or in the dormant season just before bud-break, using peach seedlings in their first year of growth, and budded varietal stocks (peach, plum or cherry) in the first growing season after budding. One infective

bud is sufficient for trees of this age, but if the trees are larger, it is advisable to increase the dosage either by multiple budding or by grafting.

Infection usually takes place if contact between living bud and stock is maintained until callusing starts, usually a matter of 10–14 days, whether the infective bud grows or dies later on.

Let us now turn our attention to some of the diseases found in Ontario—starting with the complex which has concerned us most, namely, the sour cherry yellows and necrotic ring spot and its possible relationship with prune dwarf and tatter leaf of sweet cherries.

Prune dwarf occurs naturally in sweet cherry, plum and, possibly, peach (the rosette mosaic of Michigan); necrotic ring spot on sweet and sour cherry and some plum varieties, and cherry yellows on sour cherry.

On peach, prune dwarf produces practically no shock symptoms; chronic symptoms are shortening of internodes and wavy-leaf margins. Shock symptoms of necrotic ring spot on peach are delayed foliation with death of buds and die-back of twigs; there are no chronic symptoms. Cherry yellows on peach combines the shock symptoms of necrotic ring spot with the chronic symptoms of prune dwarf.

On sour cherry, prune dwarf produces shock symptoms (delayed leafing, etching, and sometimes necrotic spotting) and no chronic symptoms. Necrotic ring spot produces shock symptoms of the same type but of a more severe form and no definite chronic symptoms. Yellows produces the same shock symptoms as necrotic ring spot, but chronic symptoms are the appearance of yellow leaves in mid-June followed by leaf drop.

On sweet cherry, prune dwarf and necrotic ring spot cause no symptoms or only very slight ones. Yellows causes similar shock symptoms to necrotic ring spot, chronic symptoms being small rings with necrotic centres.

On Italian prune, chronic symptoms of prune dwarf are strap leaves with a pebbled surface, dwarfing of plant, and abortive pistils. Shock symptoms of necrotic ring spot are small angular necrotic flecks on first leaves; no chronic symptoms. Yellows gives shock symptoms similar to those produced by necrotic ring spot and chronic symptoms like prune dwarf.

It will be seen that the reaction of cherry yellows is more or less the combined reactions of prune dwarf and necrotic ring spot, suggesting that yellows may be due to simultaneous infection with these two viruses. Cross immunization tests are in progress to see if cherry yellows will give protection against prune dwarf and necrotic ring spot.

Tatter leaf occurs on sweet cherry. It is fairly widespread in the Niagara district and there is evidence of natural spread. On peach there is no die-back. Shock symptoms are slightly delayed foliation, and chlorotic spots and rings. Chronic symptoms are a faint mosaic and oak-leaf patterns on a few scattered leaves. On sour cherry shock symptoms are much like those of necrotic ring spot. Chronic symptoms are dwarfing of the tree with premature defoliation and sometimes leaf yellowing and leaf drop in midsummer. On sweet cherry there are no shock symptoms. Chronic symptoms are generally oak-leaf patterns and interveinal necrosis leading to tattering on the first leaves. Leaves formed later in the season are normal. Varieties react differently; in some, the crop may be nearly normal, in others worthless. Die-back sometimes occurs in Elkhorn and Seneca. On Italian prune there are no shock symptoms and no chronic symptoms (or only faint patterns).

All four diseases, prune dwarf, necrotic ring spot, cherry yellows and tatter leaf, produce a slight crop reduction in any one year, but the effect is cumulative.

There is now considerable evidence suggesting that the viruses causing *X*-disease of peach, Western *X* and some forms of little cherry disease are closely related if not identical.

VIRUS DISEASES OF CACAO

Summary of talk by A. F. POSNETTE, *East Malling Research Station, Kent*

The probable origin of swollen-shoot disease of cacao by the spread of the virus from wild plants to cacao was discussed. By transferring vectors from wild plant to cacao seedlings, natural infections have been demonstrated. *Cola chlamydantha*, in particular, has been found infected in forest several miles from cacao. The elimination of the wild host plants is difficult because of their size as mature trees. Although control of spread from cacao to cacao is relatively easy by roguing, re-infection from wild hosts may occur.

The resistance of cacao to virus infection has been studied. No plants resistant to infection have been found among survivors in devastated farms, but several which carry a mild almost symptomless strain of the disease which protects them against infection with the virulent strain have been found and propagated. Among new introductions from Brazil, now being tested, there may be types partially resistant to infection by the vector. Although valuable for replanting, resistant types would not help to control the disease in the existing plantations, and this is the main problem.

A map of southern England was shown with a map of the devastated area of the Gold Coast superimposed on it. The devastated area coincided with an area which stretched from Letchworth to Reigate, including all of greater London. Since cacao is practically the only cash crop grown in the Gold Coast, the situation cannot be paralleled in Britain. Even if all orchard crops, or all straw crops were wiped out in south-east England the crisis would hardly be comparable with that in the Gold Coast.

The relationship between the virus and its mealybug vector was discussed and graphs were shown indicating the rise in the infection rate of test plants with increasing number of insects and with time of test feeding up to 1 hr. The infection rate rises with duration of infection feeding up to 10 hr. when a drop occurs if the insects were starved beforehand. If the insects are not starved, the infection rate in the test plants rises more slowly and there is no drop at 10 hr. This difference is not explained by the relative settling rates of starved and unstarved insects.

The virus is non-persistent in the feeding vector, so that only rarely can two plants be infected in succession. The virus can, however, persist for over 24 hr. if the vector is starved after the infection feed.

When feeding times and response to starvation of the vector and the persistence of the virus in the vector are considered, it appears that this cacao virus does not behave like a typical persistent or like a typical non-persistent virus.

Lantern slides were shown illustrating:

(i) The conditions under which cacao is usually grown in West Africa and the effects of swollen-shoot disease in defoliating and killing cacao trees.

(ii) The symptoms of the most virulent virus (1 *A*) in three phases of infection—preliminary (vein clearing), acute (mosaic) and chronic (vein banding)—the leaf symptoms of a milder virus (1 *C*) were shown for comparison.

(iii) The technique of infecting the seed by means of the vector, and a young seedling showing symptoms at a stage early enough to have a cotyledon still attached.

(iv) The effect of virus 1 *A* on the root system, causing suppression of root development and necrosis.

(v) The alternative host plants (wild forest trees) of the virus and of the vector.

MR S. H. CROWDY described and illustrated by slides an unusual condition of plum, referred to as 'stunt'. Diseased trees had a weeping habit and the new shoots were short, with short internodes and prominent nodes. The branches were weak and brittle, and affected trees tended to flower and fruit early. The weakness of the branches arose from a failure to form

a normal amount of xylem, rather than from abnormality of the xylem itself, as was the case with apple rubbery wood.

The disease occurred on the varieties Bountiful, Giant Prune and Evesham Wonder in different parts of the country.

Infected plums budded on seedling peach had produced stunting of the lateral shoots and slight leaf symptoms on the peaches. Plums selected as healthy had, however, induced similar but less intense symptoms on peach.

DR L. C. LUCKWILL described briefly the rubbery wood and chat fruit diseases which occurred principally on the apple, Lord Lambourne. There was evidence that the diseases had developed in Lord Lambourne as a result of headworking trees of other varieties, and it was inferred that some varieties (e.g. Delicious and Court Pendu Plat) were symptomless carriers. Dr Luckwill also stated that he had transmitted these troubles by grafting and presented evidence of spread under natural conditions.

DR I. W. PRENTICE considered that virus diseases of trees were probably more common than was generally thought. Slow-spreading viruses with a restricted host range could be controlled or even eradicated by propagating from disease-free trees selected if necessary by indexing.

There was some evidence that there were severe strains of apple mosaic and a more serious view of this disease should therefore be taken. None of the common apple rootstocks appeared to be carriers of apple mosaic and it should not be difficult to produce mosaic-free trees.

REVIEWS

Vegetatio: Acta Geobotanica: International Review of Plant Sociology, Ecology and Plant Geography. Dr W. Junk: The Hague. 30 Dutch florins.

A scientific public already super-saturated with journals may be pardoned for not showing great enthusiasm when their number is added to. There has, however, been an obvious need for a journal containing contributions from the European geobotanists, whose work has so far been available to the outside world only in scattered journals and miscellaneous publications difficult to obtain. The papers included in the first three parts of *Vegetatio* are in English, French, German or Spanish.

The English title of the journal, the *International Review of Plant Sociology, Ecology and Plant Geography*, indicates its proposed scope. In his introduction, Prof. Braun-Blanquet states: 'Modern phytosociology, which has developed from classical phytogeography, after having for a long time felt its way, took an unsuspected leap forward as soon as an international understanding allowed it to give a definite meaning to certain fundamental terms which until then were too vague, to define some basic concepts, and to suggest a logical and clear system of classification of the described units of vegetation.'

The first issues have a number of papers reviewing the present state of knowledge and activity in the subject in various countries, but most of the contents deal with many aspects of phytosociology, ranging from forest ecology in Bas-Languedoc to sand, swamp and weed vegetation in an estuary in Palestine, the characterization of grasslands in the Netherlands, pollen analysis of post-glacial peats in France, and the relation between pedology and phytosociology.

International journals of this nature deserve every support as contributing to general exchange of information, correspondence and goodwill between scientists of many different countries. It is to be hoped that the papers will not be too predominantly academic, as the workers in these subjects have much to contribute to, and to learn from, the practical world.

R. O. WHYTE

Die Pilze. By Ernst Gäumann. Pp. 382 with 440 Text-figures. Basel, Switzerland: Verlag Birkhäuser. 1949. 38 Swiss francs.

This book is number nineteen of a series of text-books and monographs on the exact sciences, and is the fourth volume to be published in the section of experimental biology. It is, in effect, a revised and condensed version, in a simpler and more attractive format, of the author's *Vergleichende Morphologie der Pilze*, which was published in 1926.

Prof. Gäumann excludes bacteria and myxomycetes altogether from his conception of fungi, and regards the Olpidiaceae, Synchytridiaceae, Plasmodiophoraceae and Olpidiopsidaceae as parasitic flagellates on an evolutionary branch line distinct from the very beginning from the main line of the true fungi. These four families are therefore separated from the Phycomycetes to form a class of their own—the Archimycetes—and the true fungi are regarded as consisting of the rest of the Phycomycetes as generally understood, the Ascomycetes and the Basidiomycetes. The large group of Fungi Imperfecti is relegated to an appendix occupying just over a page. Keys are given to the Orders within each Class and to the Families within some of the Orders.

The main thread running throughout the book is the developmental history of the fungi. The author's conception of evolutionary relationships is indicated schematically for the fungus system as a whole on p. 10, and for individual Orders and Families in about a dozen other 'family trees' elsewhere. Interwoven with the central thread is an astonishing as-

semblage of facts about the morphology, biology and reproduction of fungi, all marshalled in masterly fashion to illustrate and illumine the developmental theories put forward. The mycologist and plant pathologist cannot but find in this volume a clear, concise, invaluable digest of the accumulated knowledge about fungi. They will, however, be disappointed with the meagre treatment given to the Fungi Imperfecti. After all, the book purports to deal with 'Die Pilze', and though it would be very difficult to include the imperfect fungi in the body of a work designed primarily to discuss evolutionary tendencies, the appendix could have been greatly expanded with advantage, and especially by one whose deep insight into and understanding of the part played by fungi in plant pathology is so warmly appreciated. Over 800 references to original work are cited, and there are 440 line drawings, photographs and diagrams. Only a few of these are original, but the majority have been newly and specially drawn for the occasion by Messrs Heinz Kern and Ernst Lehmann. W. C. MOORE

Hydrobiologia: Acta Hydrobiologica Limnologica et Protistologica. Dr W. Junk: The Hague. 40 Dutch florins.

To judge from the success of the first post-war international Congress of Limnology, held at Zürich in 1948, interest in fresh-water biology is increasing, and facilities for the publication of papers on the subject are inadequate. One of the two international journals devoted to Limnology, the *Internationale Revue der gesamten Hydrobiologie und Hydrographie* was a war casualty, and this makes the appearance of a new publication, *Hydrobiologia*, particularly welcome.

Hydrobiologia is printed in Holland under the direction of a distinguished board of editors from nine different countries. The editors include some of the most eminent of European and American limnologists, and their names afford a sufficient guarantee of the high standing of the new journal, which will publish papers on all aspects of the biology and systematics of marine and fresh-water plants and animals. The first two numbers contain papers on the biology of pathogenic bacteria, the ecology and systematics of Protozoa, rotifers, insects, and algae, as well as papers of general hydrographical interest on the penetration of light into a fresh-water reservoir and on productivity. M. PRYOR

Physiologia Comparata et Oecologia: an International Journal of Comparative Physiology and Ecology. Dr W. Junk: The Hague. 36 Dutch florins (for the volume of 4 parts).

It is the intention of this new journal to publish articles, in English, French or German, embodying original research in the fields of comparative physiology and ecology of Vertebrates and Invertebrates. It will also include investigations in the general field of animal biology, embracing such topics as nutrition, feeding, mechanisms, locomotion, reproduction, hibernation and marine biology. Articles dealing exclusively with applied biology will not be accepted; nor will contributions on cellular, medical or veterinary physiology and animal psychology unless they make some definite contribution to the field of comparative physiology. The first two parts of Volume 1 have now appeared. They are excellently printed and produced, with good illustrations. The papers they contain range over such diverse subjects as X-ray studies on the movements of the hen's intestine, the thermal preferendum in insects, muscle innervation and synaptic transmission in Crustacea, respiratory movements in the developing chicken, and breeding hares in captivity. Most of the papers in these two numbers are in English and are of a high standard. V. B. WIGGLESWORTH

The Avian Egg. By ALEXIS L. ROMANOFF and ANASTASIA J. ROMANOFF. Pp. 918. New York: J. Wiley and Sons; London: Chapman and Hall, Ltd. $14.

In the course of preparing this book, the authors reviewed 15,000 publications and the list of references contains over 2600 titles. These facts, coupled with the knowledge that twenty

years have been spent on its preparation, go some way to indicating the thorough nature with which the main subject—the hen's egg prior to activation—has been studied. Prof. Romanoff and his wife are to be congratulated on bringing together studies which are scattered throughout the literature of practically every branch of science. It is unlikely that any other article of diet can have so large a scientific literature.

Without making this review unwieldy, it is impossible to give a full statement of the book's contents. The following remarks do no more than indicate the three major divisions. Under the title 'Morphogenetic Expression' are covered all aspects of laying, external characteristics, structure, formation and abnormalities. The section 'Biophysicochemical Constitution' describes the chemical composition, physico-chemical and biological properties. Finally 'Bioeconomic Importance' covers food values, preservation and industrial uses from the making of ice-cream to the preparation of vaccines. There is little doubt that this work provides an admirable source for those in search of information on eggs.

Whilst the title speaks of the 'Avian Egg', the book is almost wholly devoted to studies on the egg of the domestic hen with some references to the eggs of other domestic birds. Information on the eggs of wild birds forms a negligible part of the book. No doubt the economic importance of the hen's egg and its availability have been responsible for this avalanche of knowledge. In the reviewer's opinion, however, a greater use will have to be made of the comparative method before the full value of this vast collection of data can be realized.

J. B. CRAGG

OBITUARY NOTICE

SIR JOHN FRYER, K.B.E., F.R.S.

The death of Sir John Fryer on 22 November 1948 deprived agricultural science in general and this Association in particular of a distinguished man, whose wide knowledge, tactful administration and unstinted service, made him outstanding in a most varied and arduous sphere of work. Those who were privileged to know him at all intimately saw by turns the careful and painstaking naturalist, the keen angler and sportsman, the field biologist, the official adviser, the director of research, the chairman of committee, the able writer, the helpful and informed administrator, and the considerate friend whose great personal charm made private conversation a pleasurable experience.

John Claud Fortescue Fryer was born in 1886 near Chatteris, where his family had farmed for several generations. Both his father and his grandfather had been collectors and field naturalists so it was not surprising that as a boy he soon learned to know intimately the natural history, the birds, insects and flowers of the fenlands of East Anglia. His interest in living things continued to develop whilst he was at school at Rugby and it was expected that he would do well at Cambridge University. A member of Gonville and Caius College, he took a first class in the Natural Sciences Tripos and shortly afterwards was elected a fellow of his College.

As a member of the Percy Sladen biological expedition, Fryer visited Aldabra Island in the Indian Ocean between Zanzibar and Cape Amber, the extreme northern tip of Madagascar, and later he wrote an interesting account of the flora and fauna of this remote island. He also spent some time in Ceylon, and became interested in the genetics of certain butterflies.

In 1914, Fryer was appointed entomologist to the Board of Agriculture and his was the first full-time appointment to this post. During the war years that followed the selection of Fryer was seen to be justified, for he proved himself an able and balanced adviser and a man of exceptional foresight. His work at the Board (later the Ministry) of Agriculture, brought him into close contact with men like Daniel Hall, Thomas Middleton, George Stapledon and William Lobjoit, and he had an opportunity of contributing to the food-production policy and to the planning of future agricultural and horticultural education and research. In the post-war period he did much towards furthering the work of the fruit research stations at Long Ashton and East Malling, and the establishment of the glasshouse research station at Cheshunt.

One of his first actions at the Board of Agriculture was to enlist the voluntary services of biologists at the universities and agricultural colleges as recorders and advisers, in this way anticipating the establishment of a co-ordinated specialist advisory service covering the whole country.

During the 1914–18 war little could be done to develop the new advisory service, but with the aid of his colleague A. D. Cotton, as mycologist, valuable work was done from Kew. In 1920, the staff moved to Harpenden where the Ministry's Plant Pathology Laboratory was established with Fryer as its first director. With the help of J. E. Collin as entomologist, and later G. H. Pethybridge as mycologist, official advisory work on pest problems was carried on, and the newly established de-centralized specialist advisory services in entomology and mycology were co-ordinated.

Fryer himself was actively interested in the entomological investigations, and with H. V. Taylor made a useful survey of basket willow growing in Britain and defined the major pest problems that confronted the industry. Other subjects that received his special attention were varietal resistance of oats to frit-fly attack, the capsid bug as a new threat to fruit growing, control of cutworms, a problem emphasized by the extension of market growing,

and Colorado beetle, now well established in Europe and likely to be introduced into Britain.

Between the wars the position of the Harpenden laboratories became firmly established as a central co-ordinating advisory bureau, and its services were sought by governments and specialists all over the world; and Fryer's personal contributions to discussions on colonial entomological problems were much valued by the colonial administrators. At home the advisory entomologists in the provinces were developing local research and advisory work, and Fryer's great personal interest in the investigations was always most valuable and stimulating. On the administrative side and in committee, Fryer's agricultural background, his specialist knowledge, and his great interest in biological research, made his opinion much sought after, and so in the course of time his services to agricultural research were widely recognized and highly esteemed. On the entomological side two good examples of his leadership are seen in the organization of team work on wireworm problems that led to the national wireworm advisory survey during the war, and in the success of the campaign to prevent the establishment of the Colorado beetle in Britain.

During the inter-war period Fryer found time to write and, in addition to many scientific reports, he produced valuable reviews of the development of pest control in this country for the *Annals of Applied Biology*, the *Journal of the Royal Agricultural Society* and for the volume of 'essays presented to Sir Daniel Hall'.

When in 1941 the Ministry of Agriculture formed the Agricultural Improvement Council to ensure that new scientific knowledge was sensibly used in agricultural practice, Fryer became secretary. The work of the Council was closely associated with that of other official bodies and with the Agricultural Research Council, and Fryer's wide experience of educational and advisory work and his knowledge of the problems of all types of agriculture ensured that the work of the Agricultural Improvement Council received immediate recognition and support.

On the death of W. W. C. Topley in 1944, Fryer was appointed Secretary to the Agricultural Research Council. It was at a time when the Council's responsibility for the future development of a national agricultural research service was imminent, and to Fryer fell the heavy task of co-ordinating, preparing and approving research programmes, supervising staffing schemes and building plans with all the attendant office and committee work.

Fryer had many active interests outside his official work. He was a Fellow of the Royal Entomological Society which he served as president. He maintained valuable collections of Microlepidoptera, Coleoptera, and Hemiptera-Heteroptera and was constantly adding to and improving them. He was an original member of the Society for the Promotion of Nature Reserves and, with H. McD. Edelsten, managed Woodwalton Fen on behalf of the Society. Fryer was elected a member of the Association of Applied Biologists in 1913 and at various times he served the Association as President, as Treasurer, and as a member of Council.

Fryer's long and distinguished services to agriculture were recognized by the award of a Knighthood in 1946; later he was elected a Fellow of the Royal Society, and Doctor of Laws, *honoris causa*, of the University of Edinburgh.

In his home at Harpenden Sir John was a charming and considerate host, and in the homes of his friends he was a welcome guest. Fishing in a mountain stream, walking over moorland, or searching for the obscure stages of insects or their host plants he was an ideal companion, and his loss is mourned by his many friends and colleagues.

The Association has lost a valued and greatly esteemed member who did much to further its work, it will all the more extend sympathy to Lady Fryer and to her son and daughter in their bereavement.

HERBERT W. MILES